

EXP#942: AMV564 FACS

11/14/17

Sample	Patient ID	EXP No.	Cycle	Timepoint	Tissue	Viability (%)	Live (cell/mL)	Total V (mL)	Total cells
1	02-004	MR921	1	Screen	BM	71.5	2.34E+05	5	1.17E+06
2	02-004	MR921	1	Day 1	PB	90.7	2.37E+05	5	1.19E+06
3	02-004	MR921	1	Day 2	PB	88.9	3.06E+05	5	1.53E+06
4	02-004	MR921	1	Day 3	PB	82.1	2.87E+05	5	1.44E+06
5	02-004	MR921	1	Day 15	PB	82.9	5.90E+05	5	2.95E+06
6	02-004	MR921	1	Day 15	BM	25	5.67E+04	5	2.84E+05
7	02-004	MR921	1	Day 29	PB	90	2.79E+05	5	1.40E+06
8	02-004	MR921	1	Day 29	BM	50.1	1.39E+05	5	6.95E+05
9	02-004	MR921	2	Day 1	PB	94.7	5.59E+05	5	2.80E+06
10	02-004	MR921	2	Day 2	PB	89.8	3.97E+05	5	1.99E+06
11	02-004	MR921	2	Day 3	PB	90.4	4.92E+05	5	2.46E+06
12	02-004	MR921	2	Day 15	PB	85.4	5.47E+05	5	2.74E+06
13	02-004	MR921	2	Day 15	BM	65.9	1.68E+05	5	8.40E+05
PBMC						82	1.26E+06	5	6.30E+06

AMV564: mAbs

Set 1: MDSC

mAb	Source	Catalog no.	Clone	Fluorophore
CD15	BDB	555401	HI98	FITC
CD124	R&D	FAB230P	25463	PE
CD14	Coulter	IM2707U	RMO52	ECD
CD11b	Coulter	A54822	Bear1	PC7
CD33	BDB	551378	WM53	APC
CD3	Biologend	344821	SK7	AF700
CD19	Biologend	363033	SJ25C1	AF700
CD20	Biologend	302322	2H7	AF700
CD56	BDB	565140	NCAM16.2	APC-R700
CD16	Biologend	302025	3G8	AF700
HLA-DR	BDB	564808	Tu169	BV421
CD45	Biologend	304035	HI30	BV510

Set 2: AML

mAb	Source	Catalog no.	Clone	Fluorophore
CD45	BDB	555482	HI30	FITC
HLA-DR	BDB	555812	G46-6	PE
CD117	Biologend	313211	104D2	PC7
CD33	BDB	551378	WM53	APC
CD123	Biologend	344821	FAB301P	AF700
CD34	BDB	562577	581	BV421
CD14	Biologend	301841	M5E2	BV510


Analysis of MDSCs mAb Set #1

Cancer Immunol Immunother (2016) 65:161–169
DOI 10.1007/s00262-015-1782-5



ORIGINAL ARTICLE

Toward harmonized phenotyping of human myeloid-derived suppressor cells by flow cytometry: results from an interim study

Susanna Mandruzzato^{1,2}  · Sven Brandau³ · Cedrik M. Britten^{4,5} ·
Vincenzo Bronte⁶ · Vera Damuzzo^{1,2} · Cécile Gouttefangeas⁷ · Dominik Maurer⁸ ·
Christian Ottensmeier⁹ · Sjoerd H. van der Burg¹⁰ · Marij J. P. Welters¹⁰ ·
Steffen Walter⁸

Cytometry Part B (Clinical Cytometry) 88B:77–91 (2015)

Review

Complexity and Challenges in Defining Myeloid-Derived Suppressor Cells

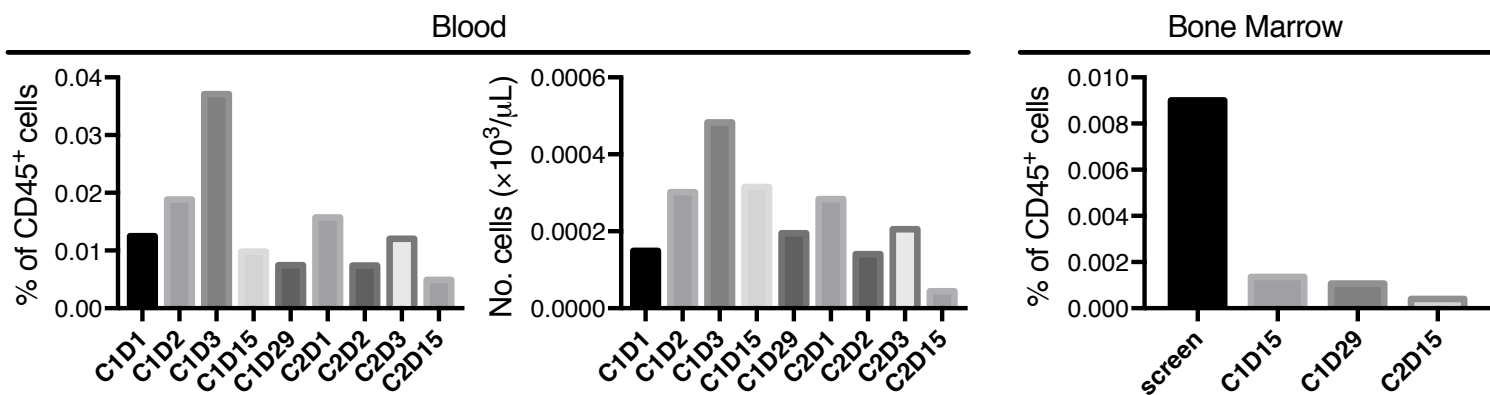
Vera Damuzzo,^{1†} Laura Pinton,^{1†} Giacomo Desantis,² Samantha Solito,¹
Ilaria Marigo,² Vincenzo Bronte,³ and Susanna Mandruzzato^{1,2*}

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University of Padova, Padova, Italy

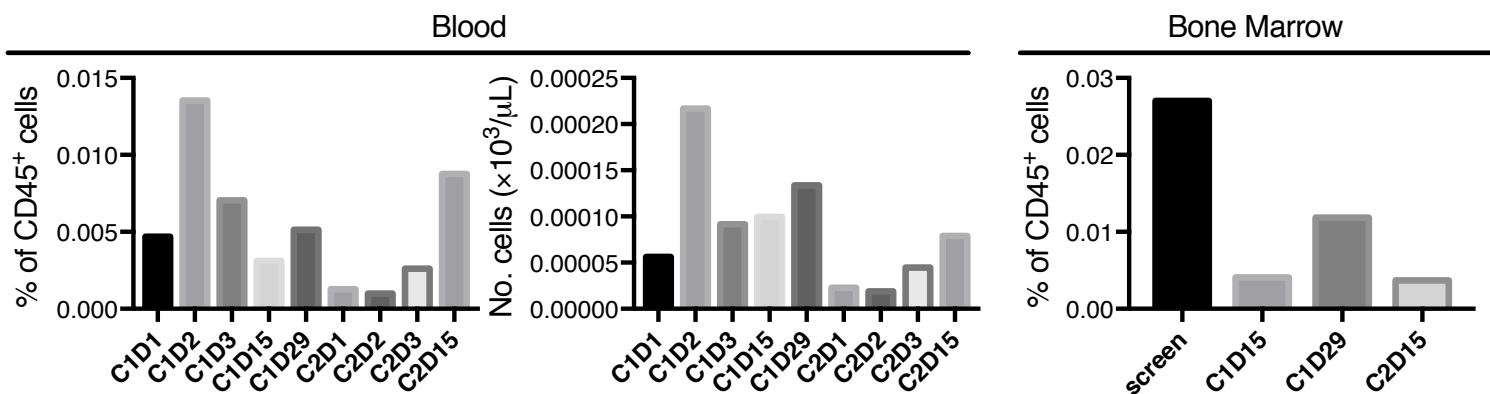
²Veneto Institute of Oncology IOV—IRCCS, Padova, Italy

³Section of Immunology, Department of Pathology, Verona University Hospital, Verona, Italy

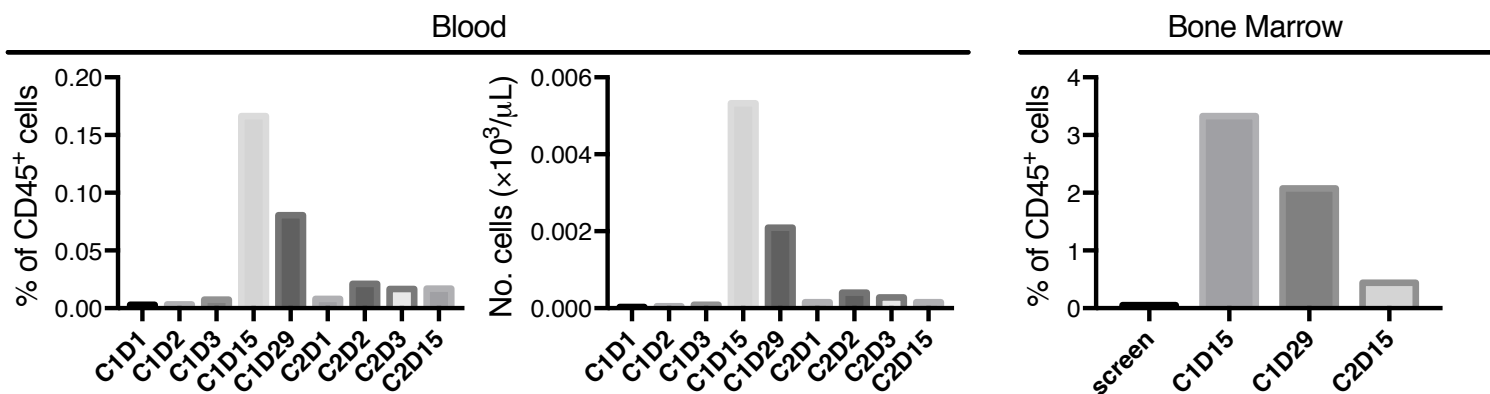
MDSC1 (CD45⁺CD14⁺CD124⁺)



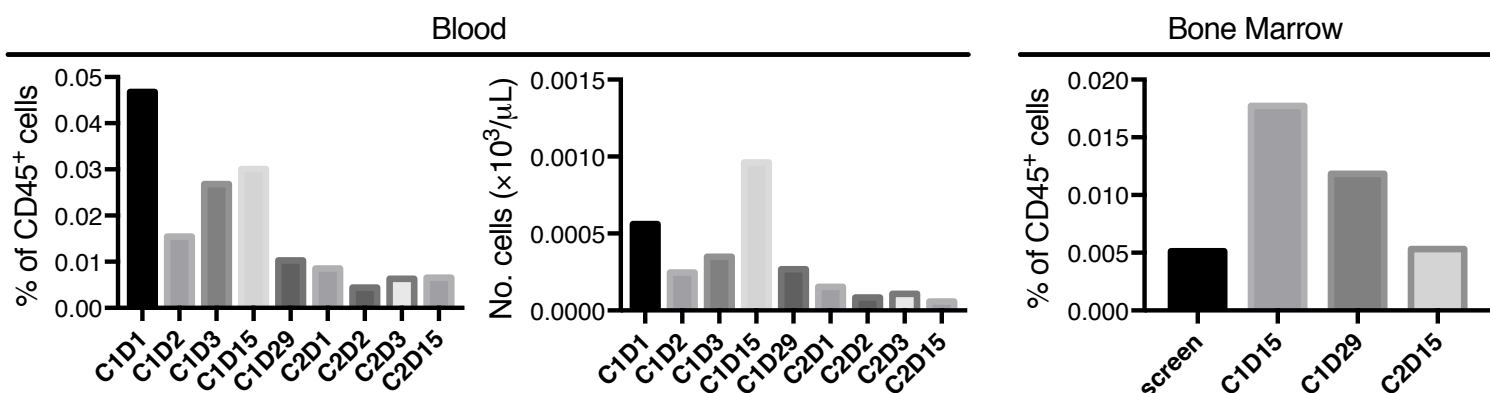
MDSC2 (CD45⁺CD15⁺CD124⁺)



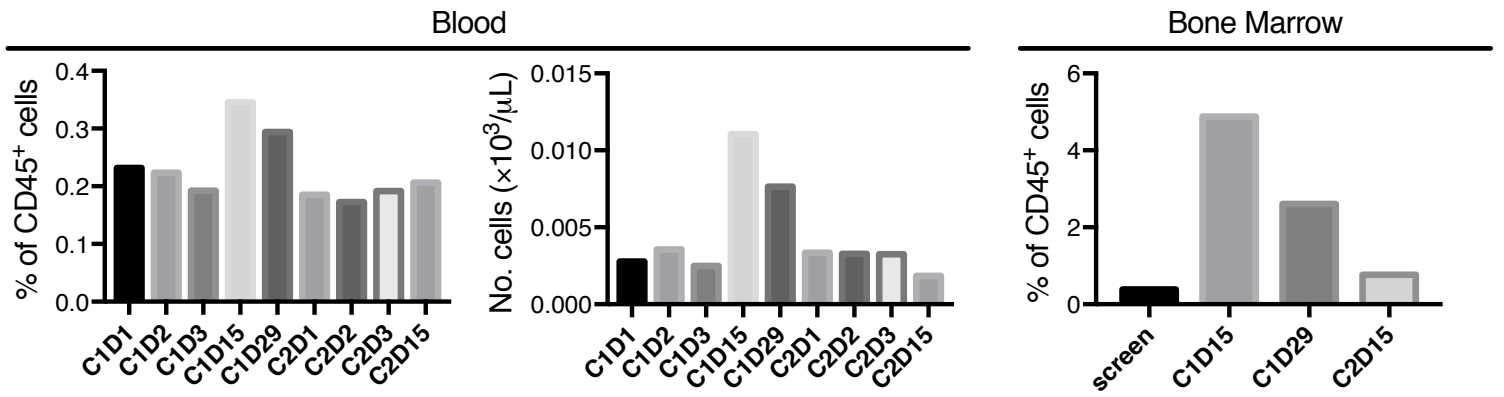
MDSC3 (CD45⁺Lin⁻HLA-DR⁻CD33⁺SSC^{hi})



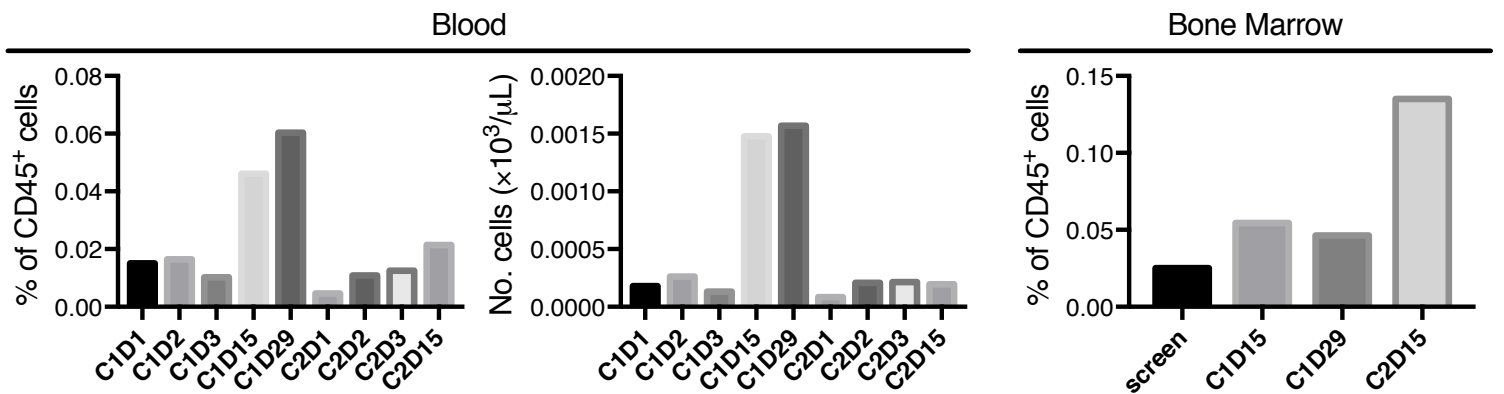
MDSC4 (CD45⁺CD14⁺HLA-DR^{lo/-})



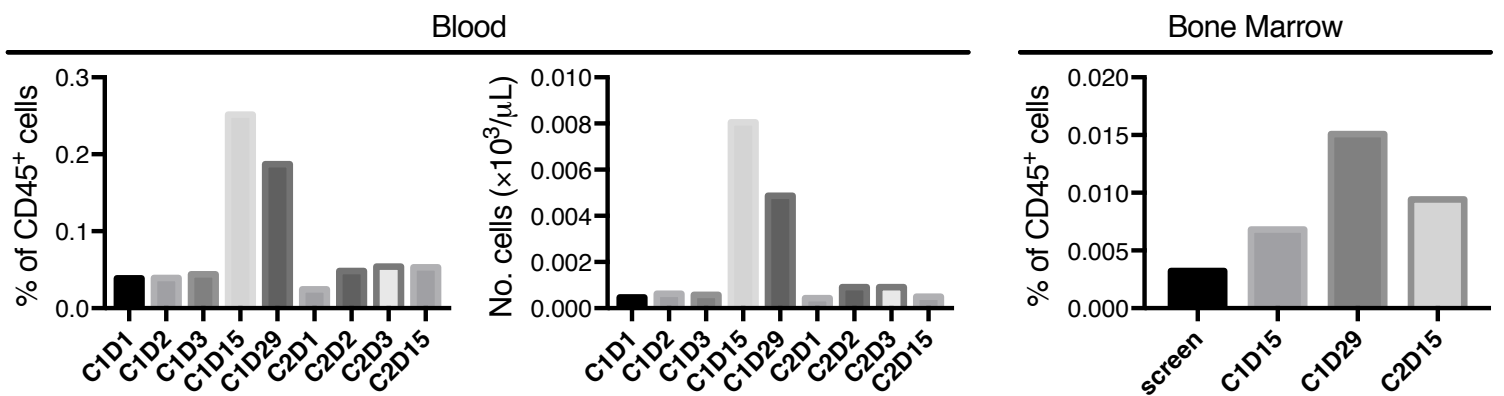
MDSC5 (CD45⁺CD14⁻CD15⁺CD11b⁺)



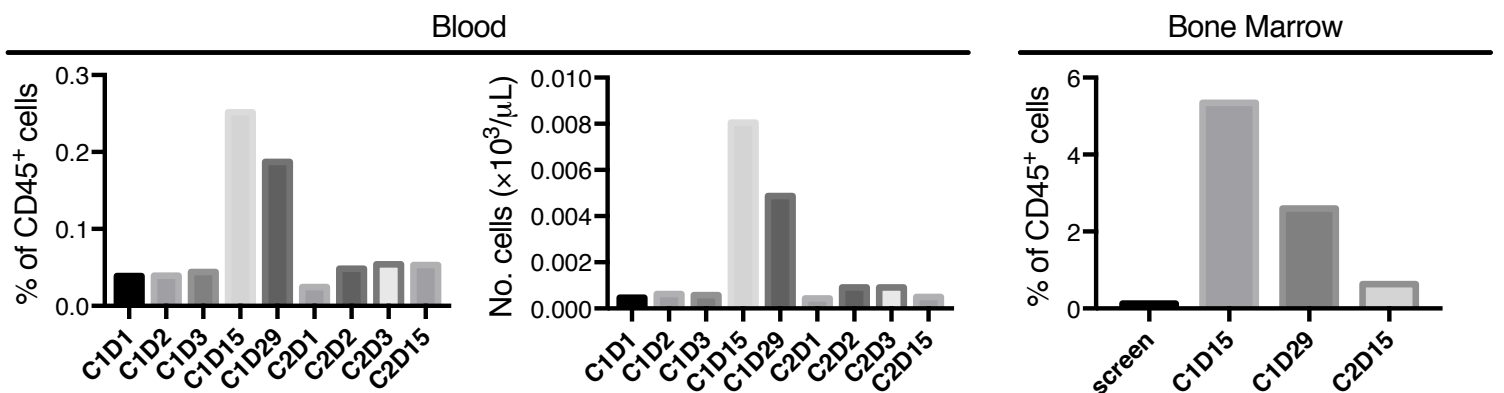
MDSC6 (CD45⁺CD15^{hi}SSC^{hi}FSC^{lo})



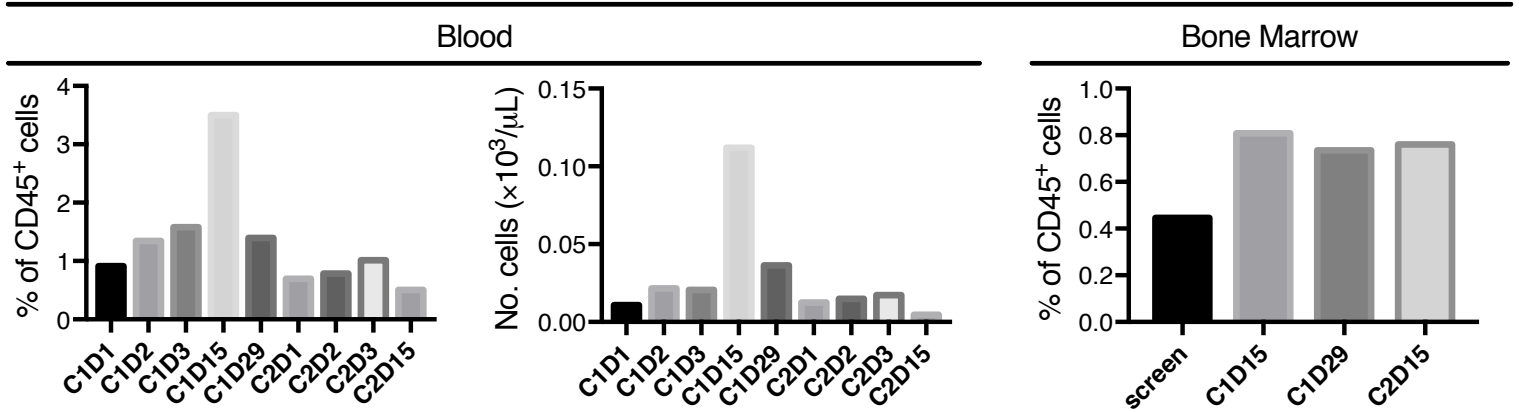
MDSC7 (CD45⁺CD14⁺CD15⁻CD33^{hi}HLA-DR^{lo/-})



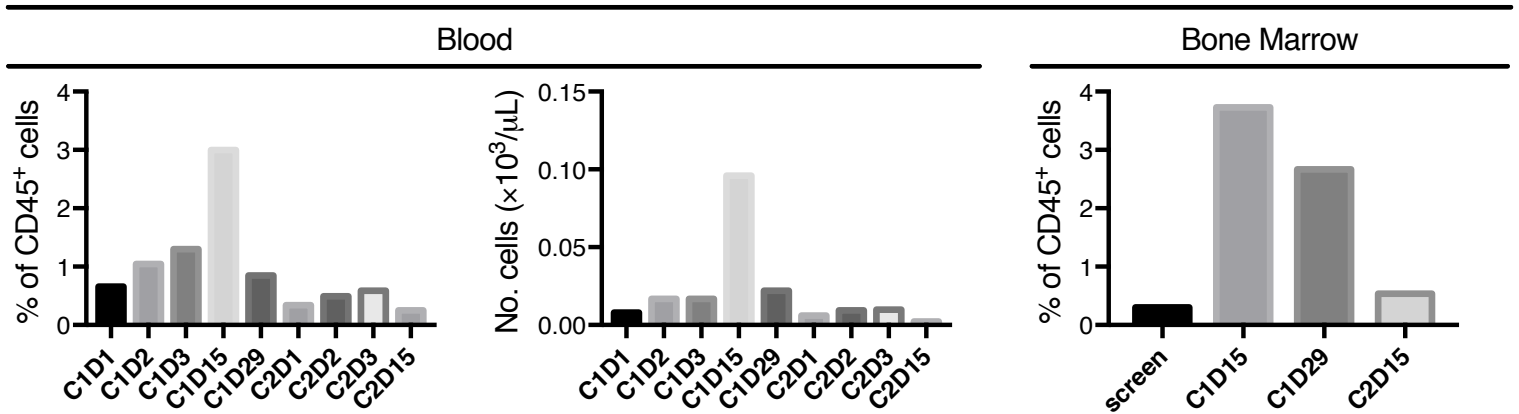
MDSC8 (CD45⁺CD15⁺CD33⁺)



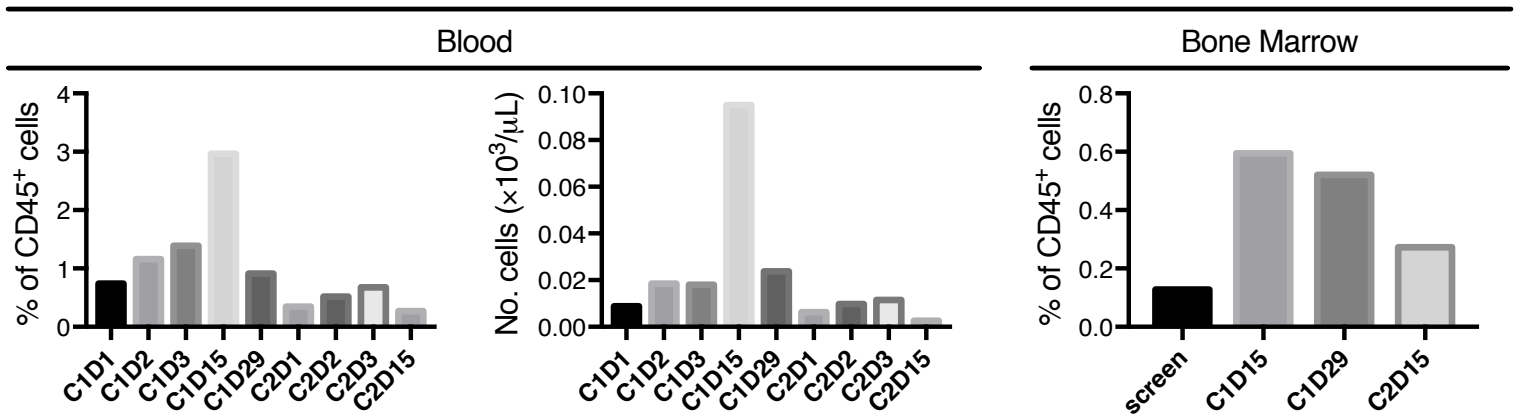
MDSC9 (CD45⁺CD14⁻CD15⁻CD33⁺)



MDSC10 (CD45⁺Lin⁻HLA-DR⁻CD11b⁺)



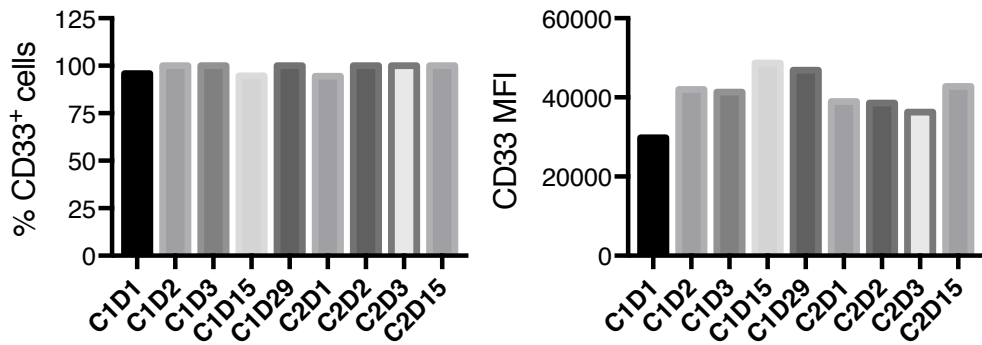
Moffitt MDSC (CD45⁺Lin⁻CD14⁻CD15⁻CD33⁺)



**Expression of CD33 on MDSCs
mAb Set #1**

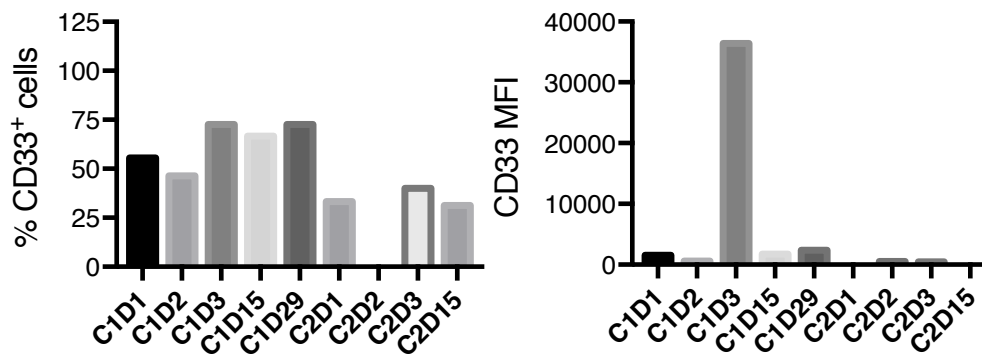
MDSC1 (CD45⁺CD14⁺CD124⁺)

Blood



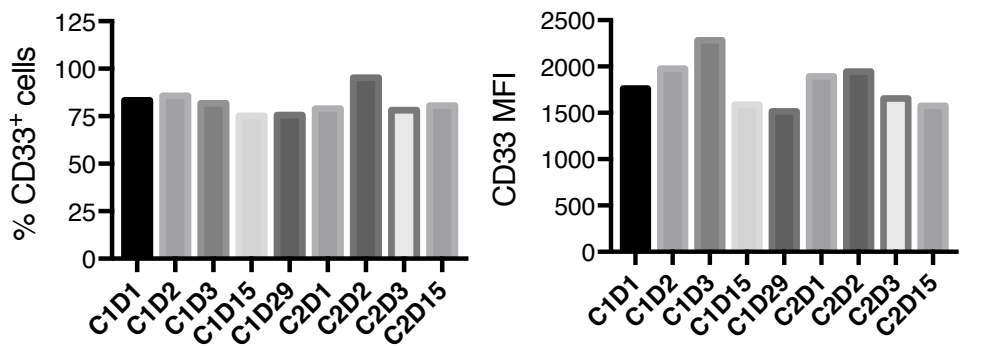
MDSC2 (CD45⁺CD15⁺CD124⁺)

Blood



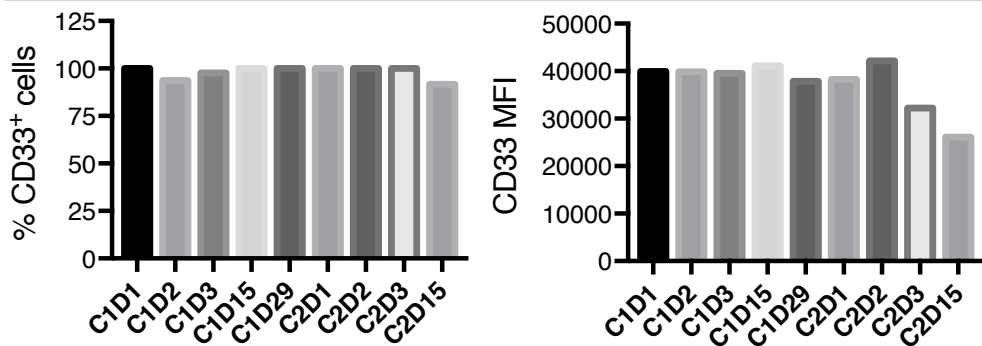
MDSC3 (CD45⁺Lin⁻HLA-DR⁻CD33⁺SSC^{hi})

Blood



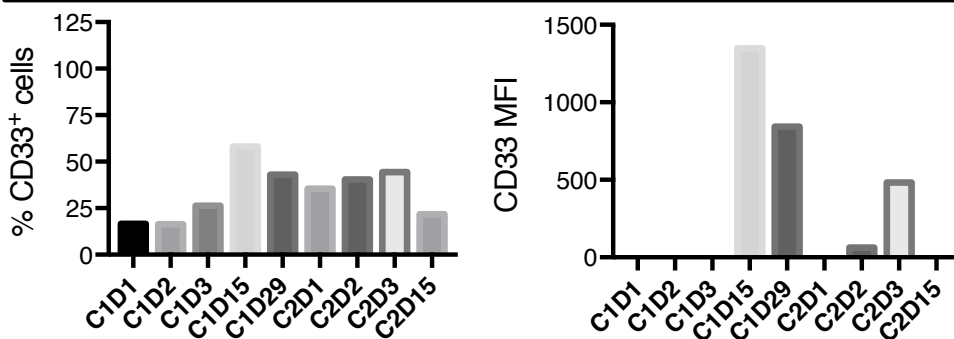
MDSC4 (CD45⁺CD14⁺HLA-DR^{lo/-})

Blood



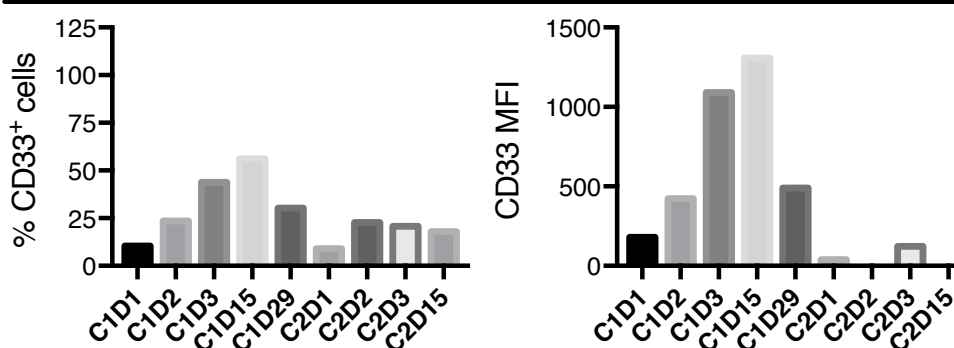
MDSC5 (CD45⁺CD14⁻CD15⁺CD11b⁺)

Blood



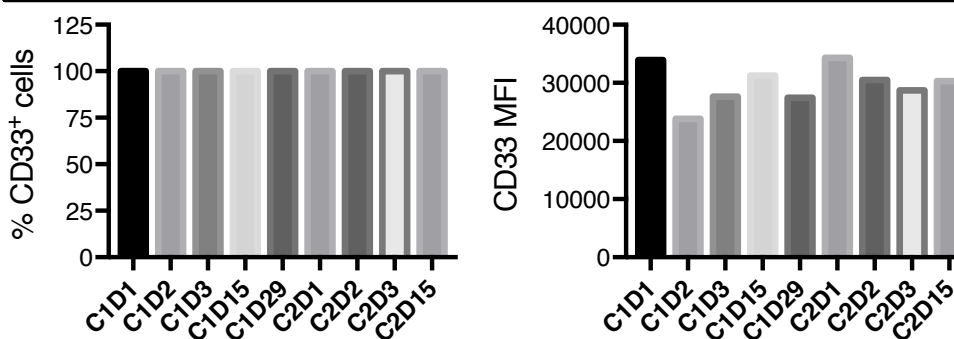
MDSC6 (CD45⁺CD15^{hi}SSC^{hi}FSC^{lo})

Blood



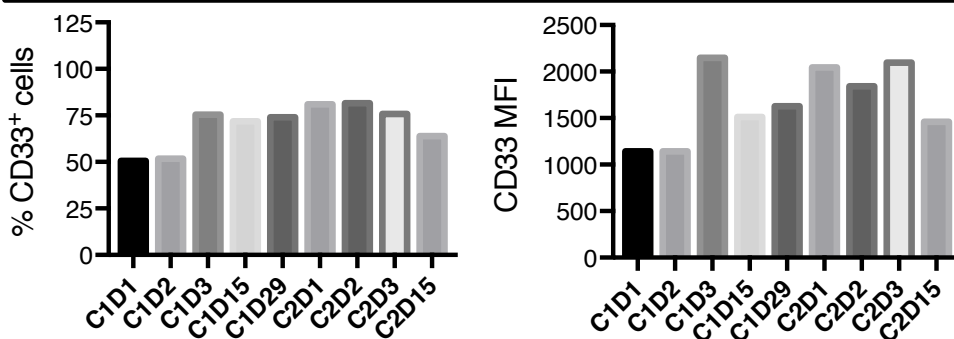
MDSC7 (CD45⁺CD14⁺CD15⁻CD33^{hi}HLA-DR^{lo/-})

Blood



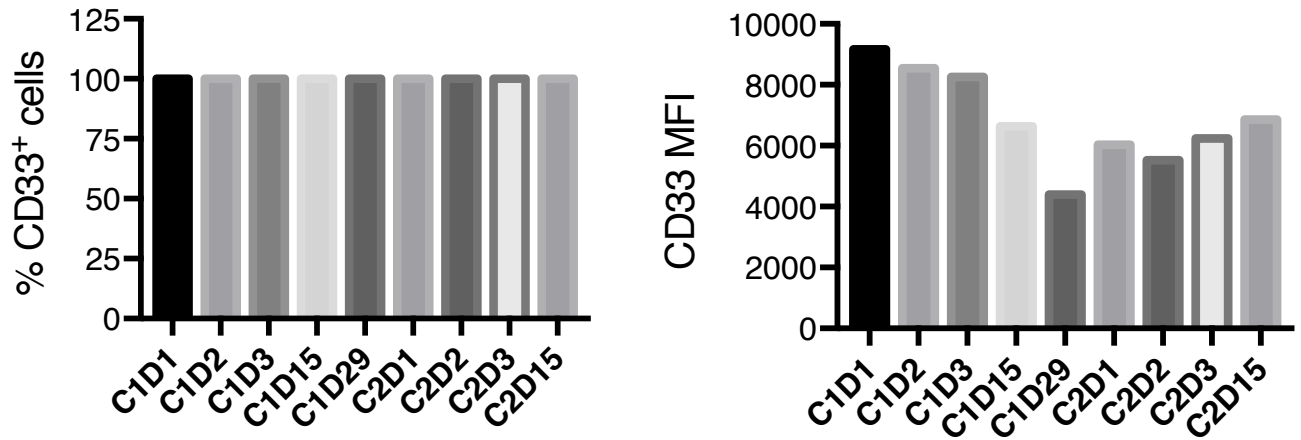
MDSC8 (CD45⁺CD15⁺CD33⁺)

Blood



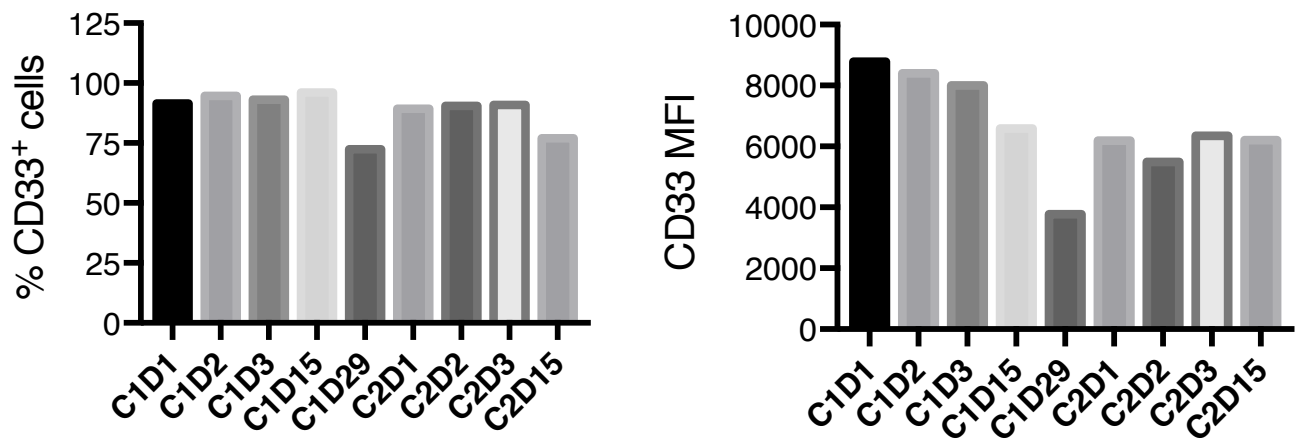
MDSC9 (CD45⁺CD14⁻CD15⁻CD33⁺)

Blood



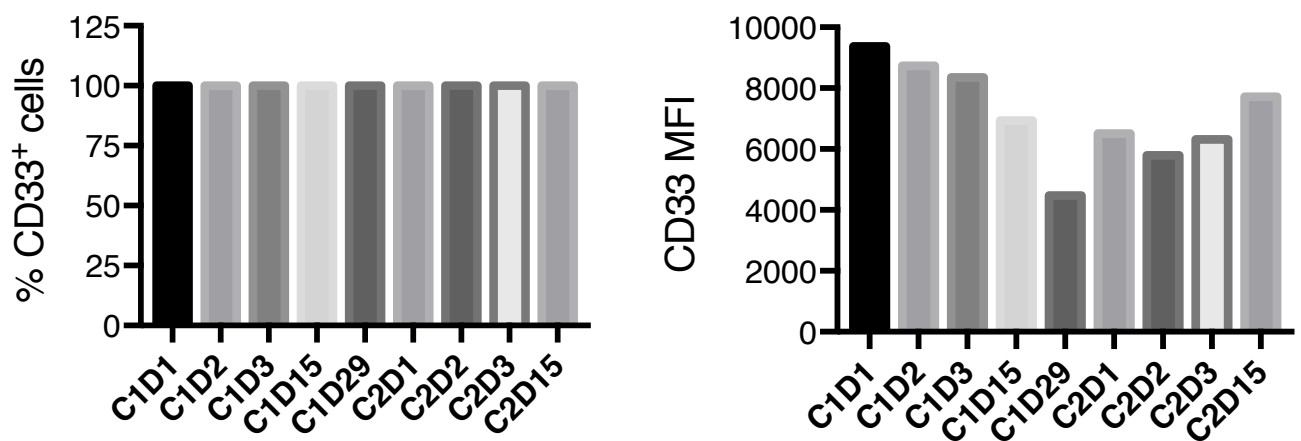
MDSC10 (CD45⁺Lin⁻HLA-DR⁻CD11b⁺)

Blood



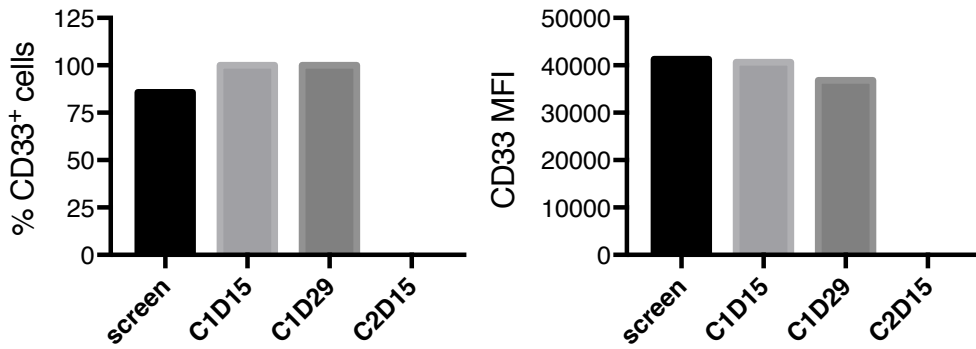
Moffitt MDSC (CD45⁺Lin⁻CD14⁻CD15⁻CD33⁺)

Blood



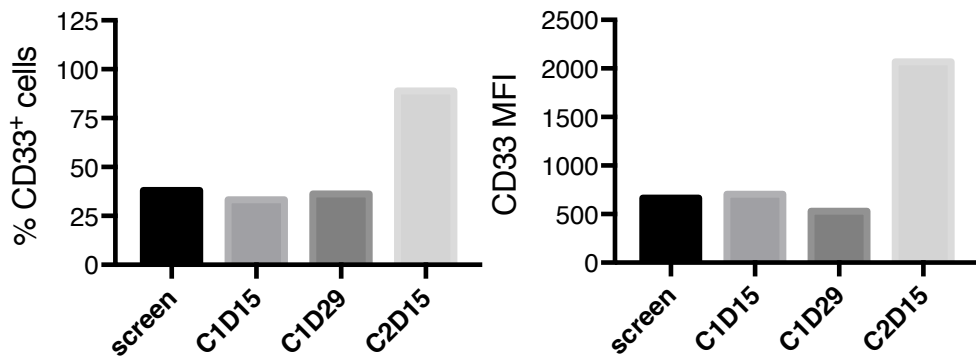
MDSC1 (CD45⁺CD14⁺CD124⁺)

Bone Marrow



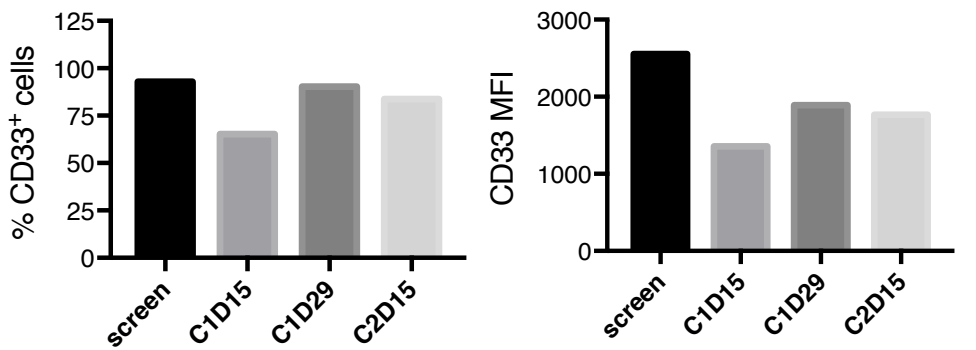
MDSC2 (CD45⁺CD15⁺CD124⁺)

Bone Marrow



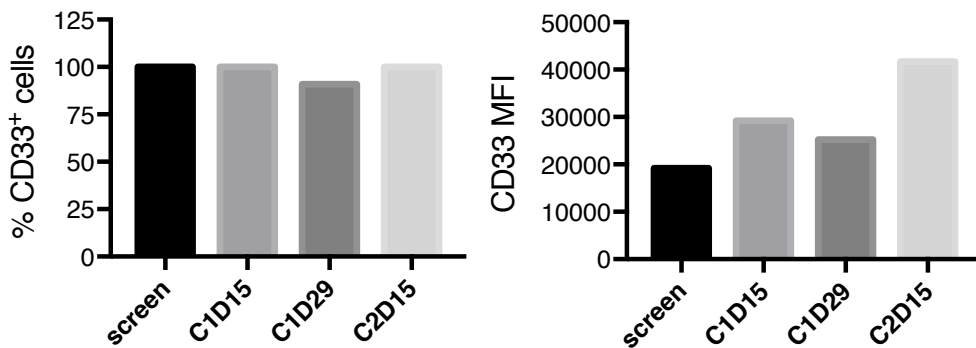
MDSC3 (CD45⁺Lin⁻HLA-DR⁺CD33⁺SSC^{hi})

Bone Marrow



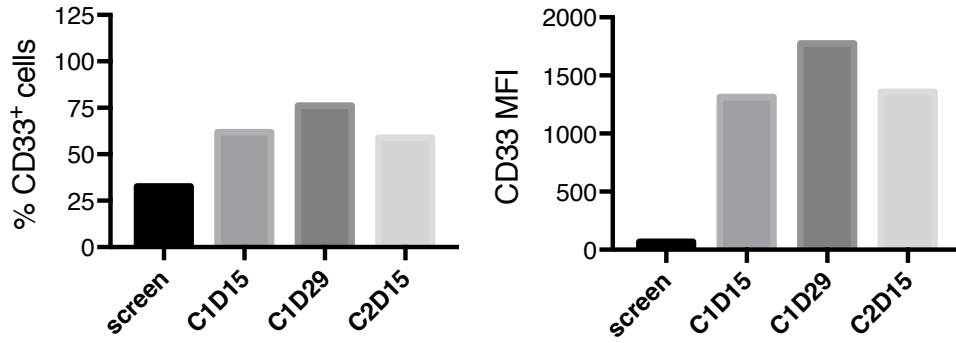
MDSC4 (CD45⁺CD14⁺HLA-DR^{lo/-})

Bone Marrow



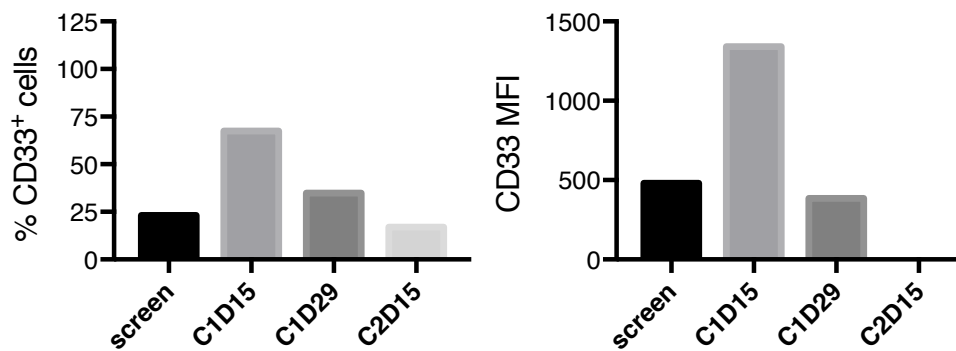
MDSC5 (CD45⁺CD14⁻CD15⁺CD11b⁺)

Bone Marrow



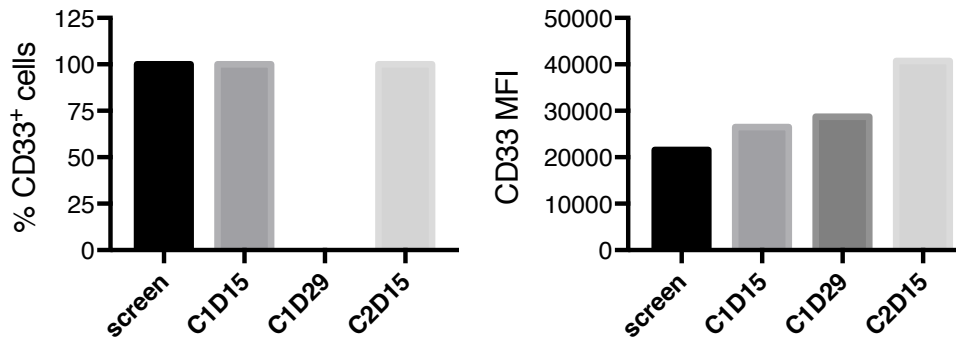
MDSC6 (CD45⁺CD15^{hi}SSC^{hi}FSC^{lo})

Bone Marrow



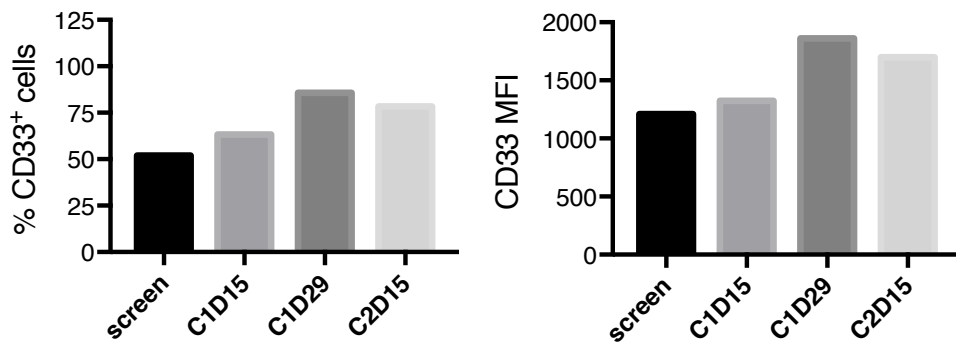
MDSC7 (CD45⁺CD14⁺CD15⁻CD33^{hi}HLA-DR^{lo/-})

Bone Marrow



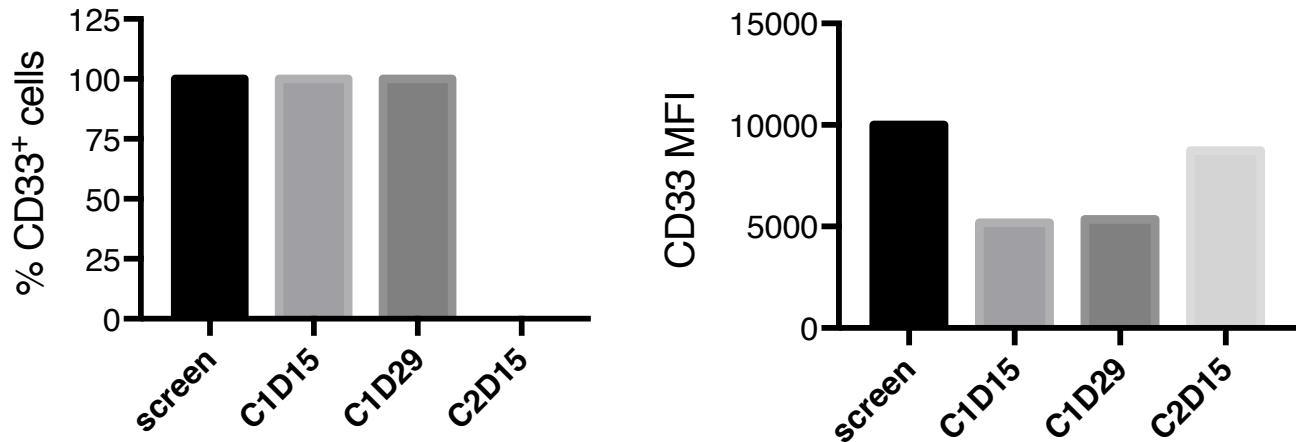
MDSC8 (CD45⁺CD15⁺CD33⁺)

Bone Marrow



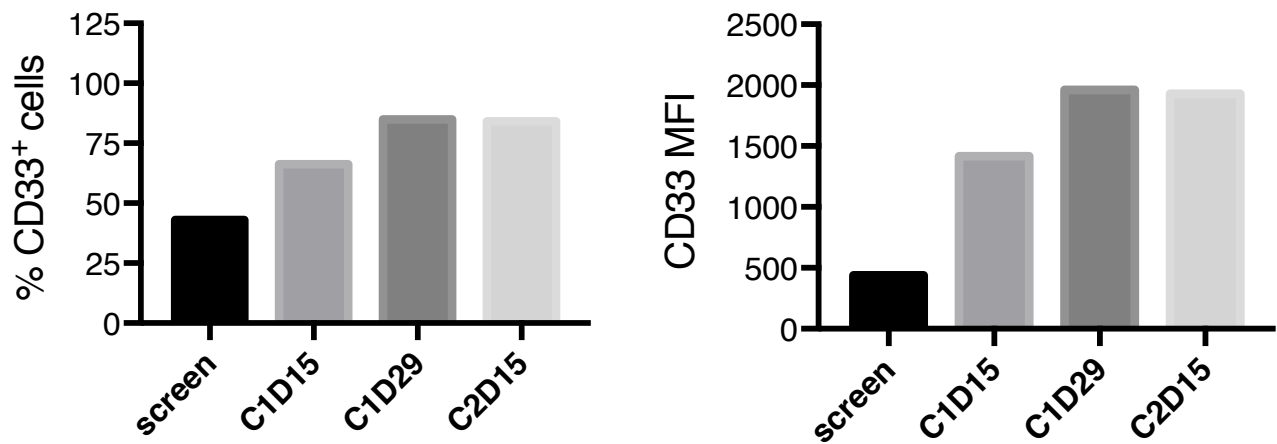
MDSC9 (CD45⁺CD14⁻CD15⁻CD33⁺)

Bone Marrow



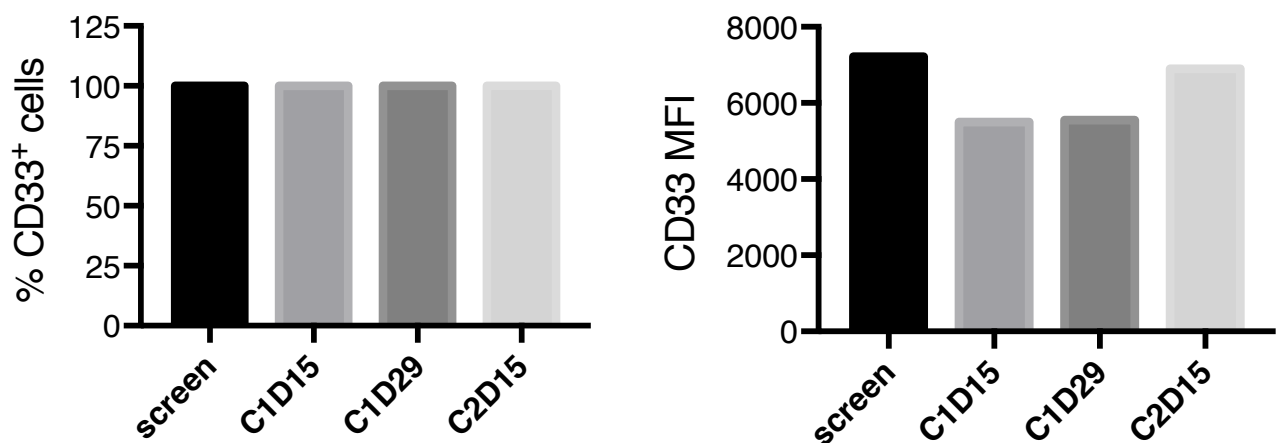
MDSC10 (CD45⁺Lin⁻HLA-DR⁻CD11b⁺)

Bone Marrow



Moffitt MDSC (CD45⁺Lin⁻CD14⁻CD15⁻CD33⁺)

Bone Marrow

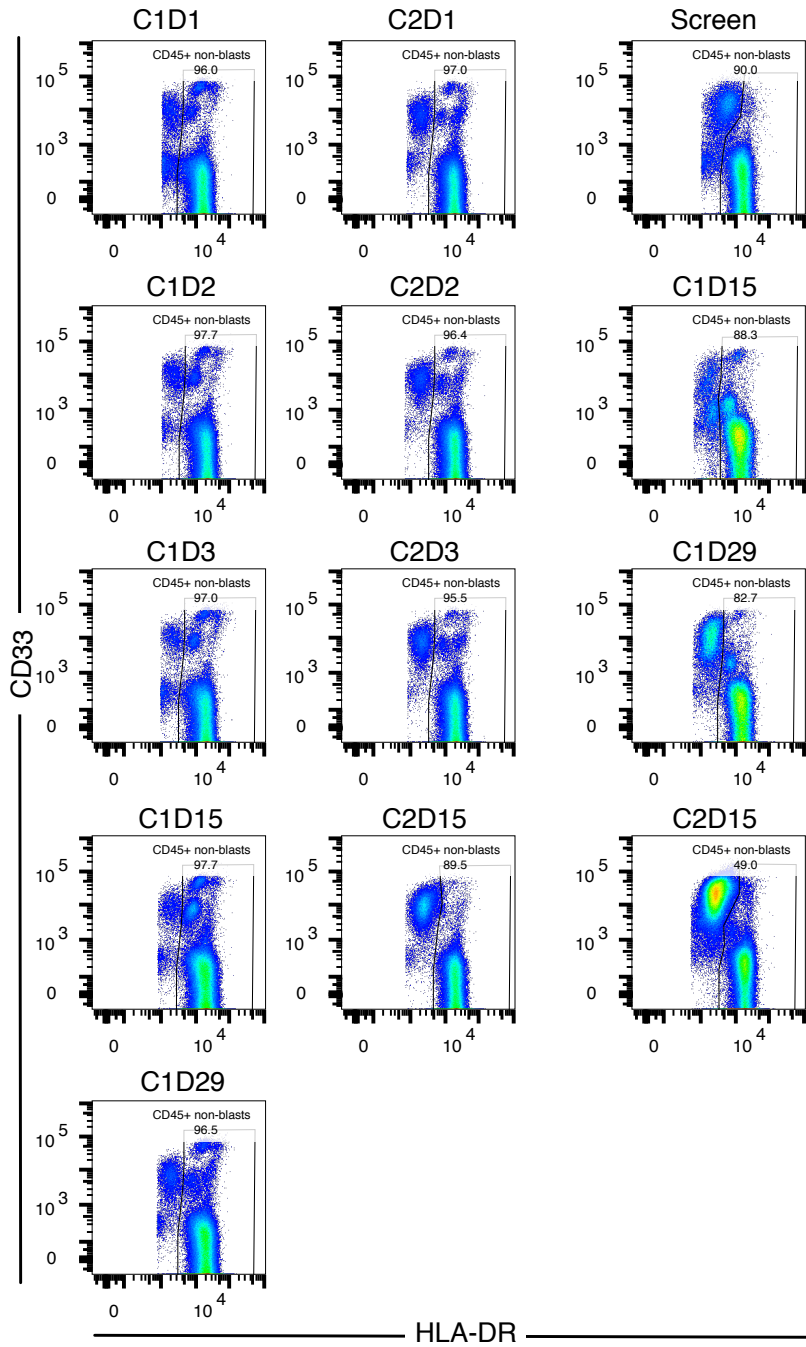


**FACS profiles of MDSCs
mAb Set #1**

Initial MDSC Gate (CD45+)

Blood

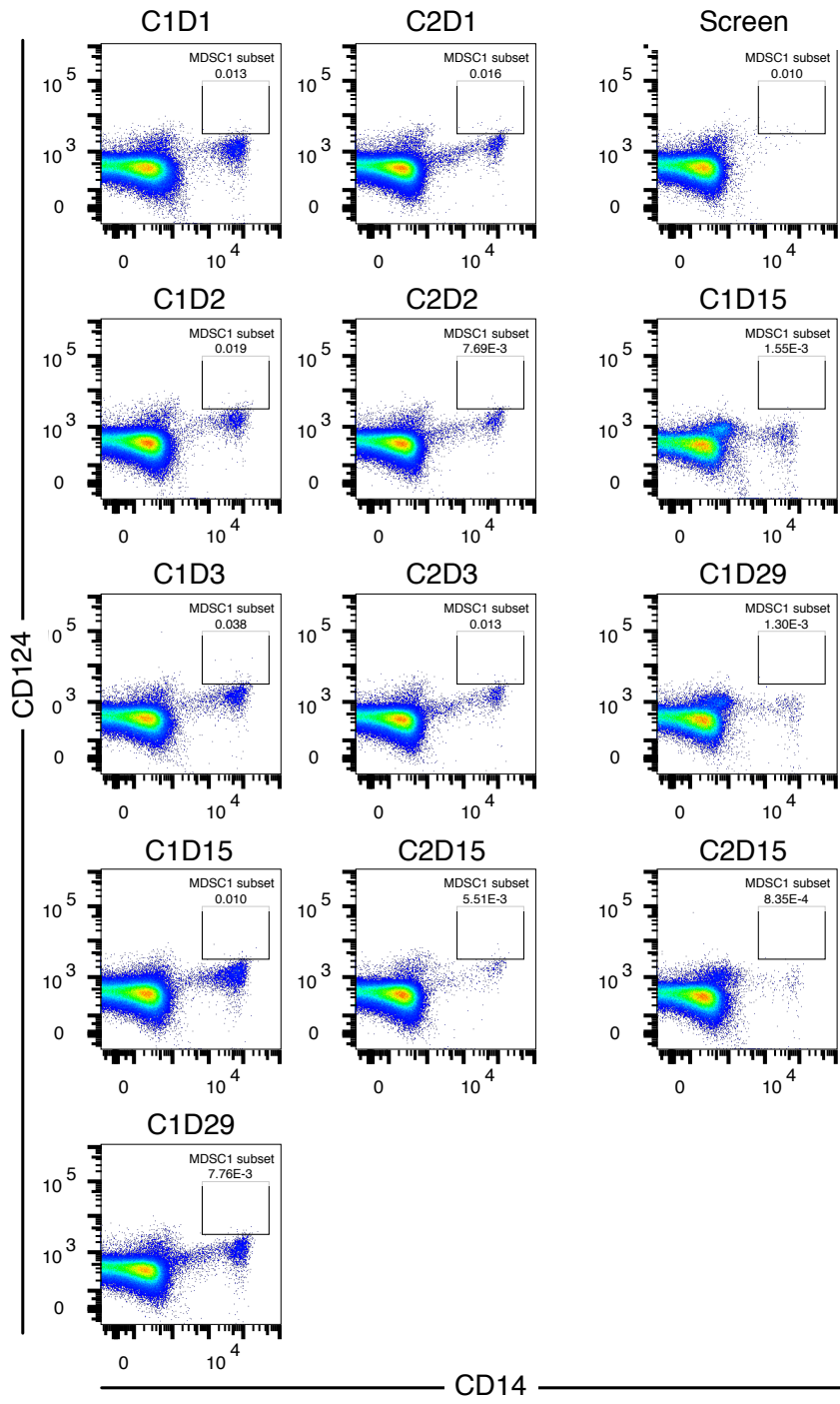
Bone Marrow



MDSC1 (CD45+CD14+CD124+)

Blood

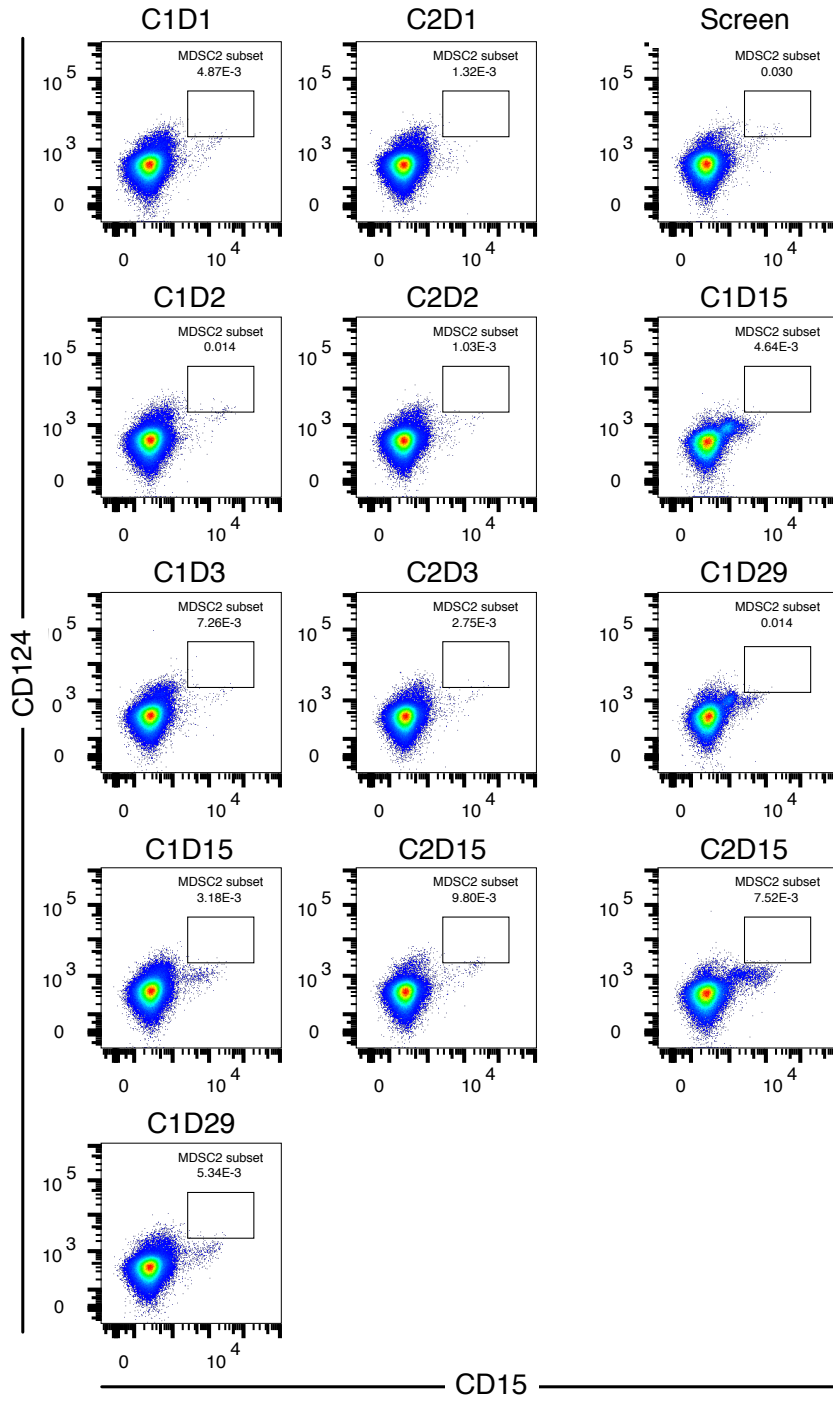
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MDSC2 (CD45+CD15+CD124+)

Blood

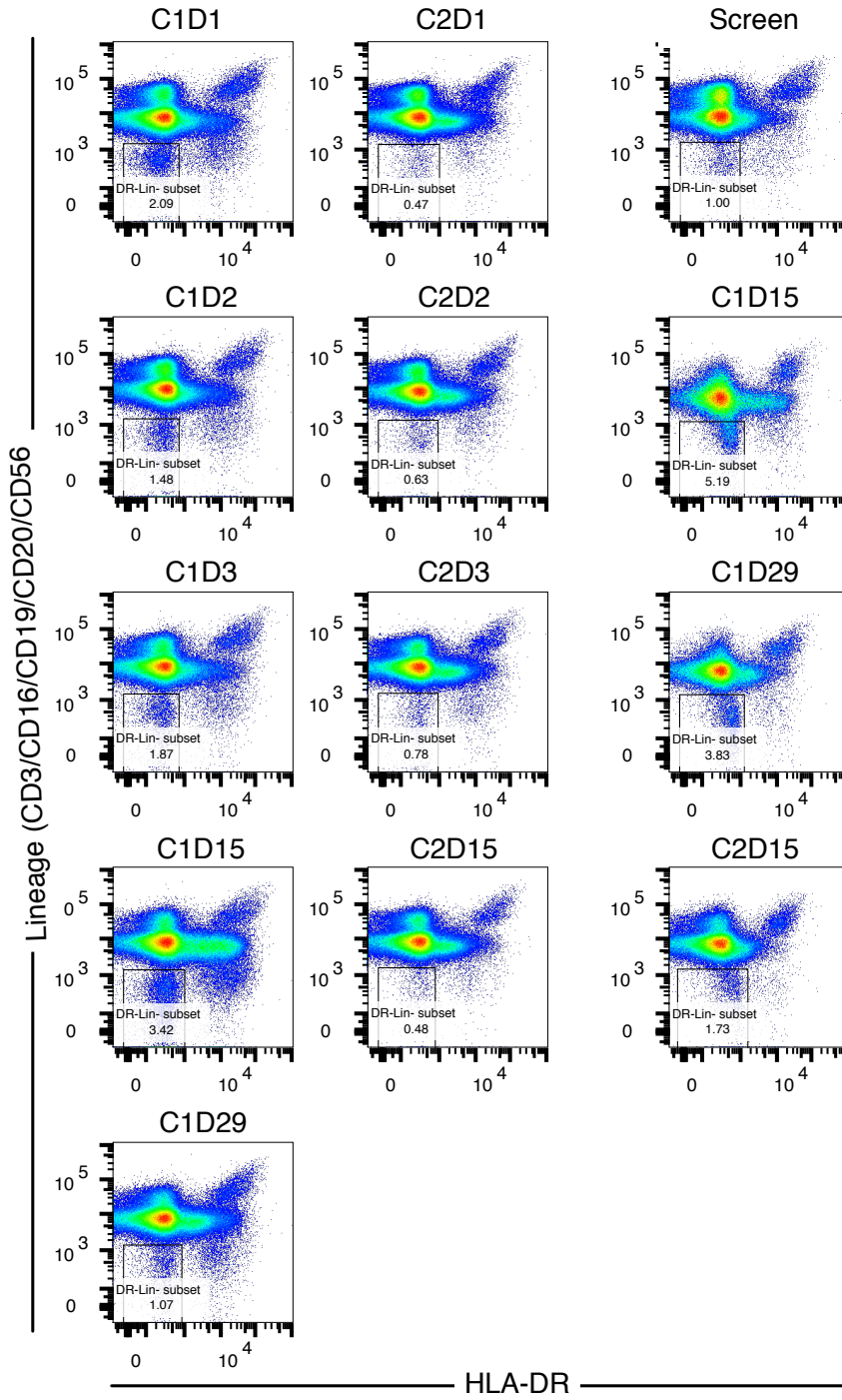
Bone Marrow



MDSC3 Base Gate (CD45+HLA-DR-Lin-)

Blood

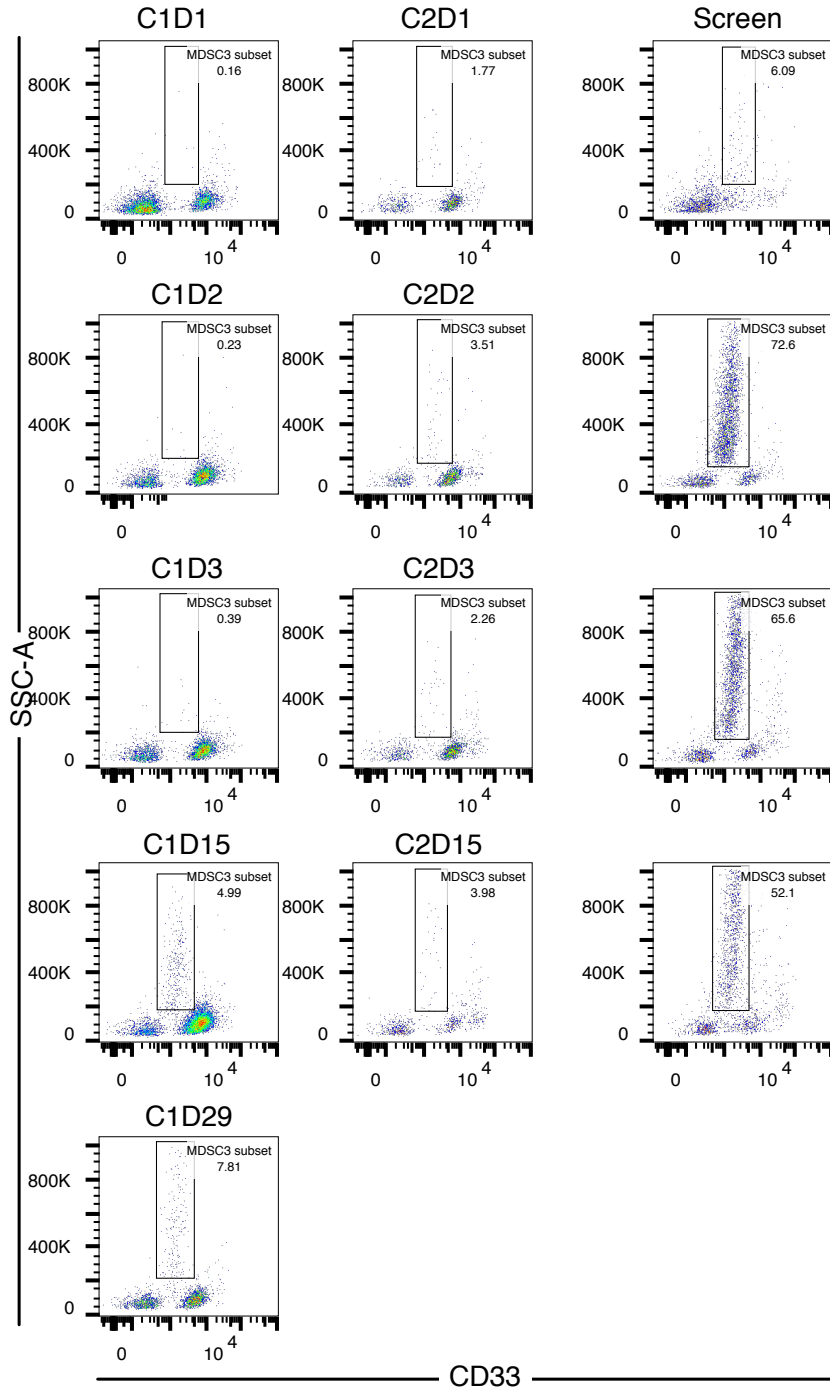
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MDSC3 (CD45+Lin-HLADR-CD33+SSChi)

Blood

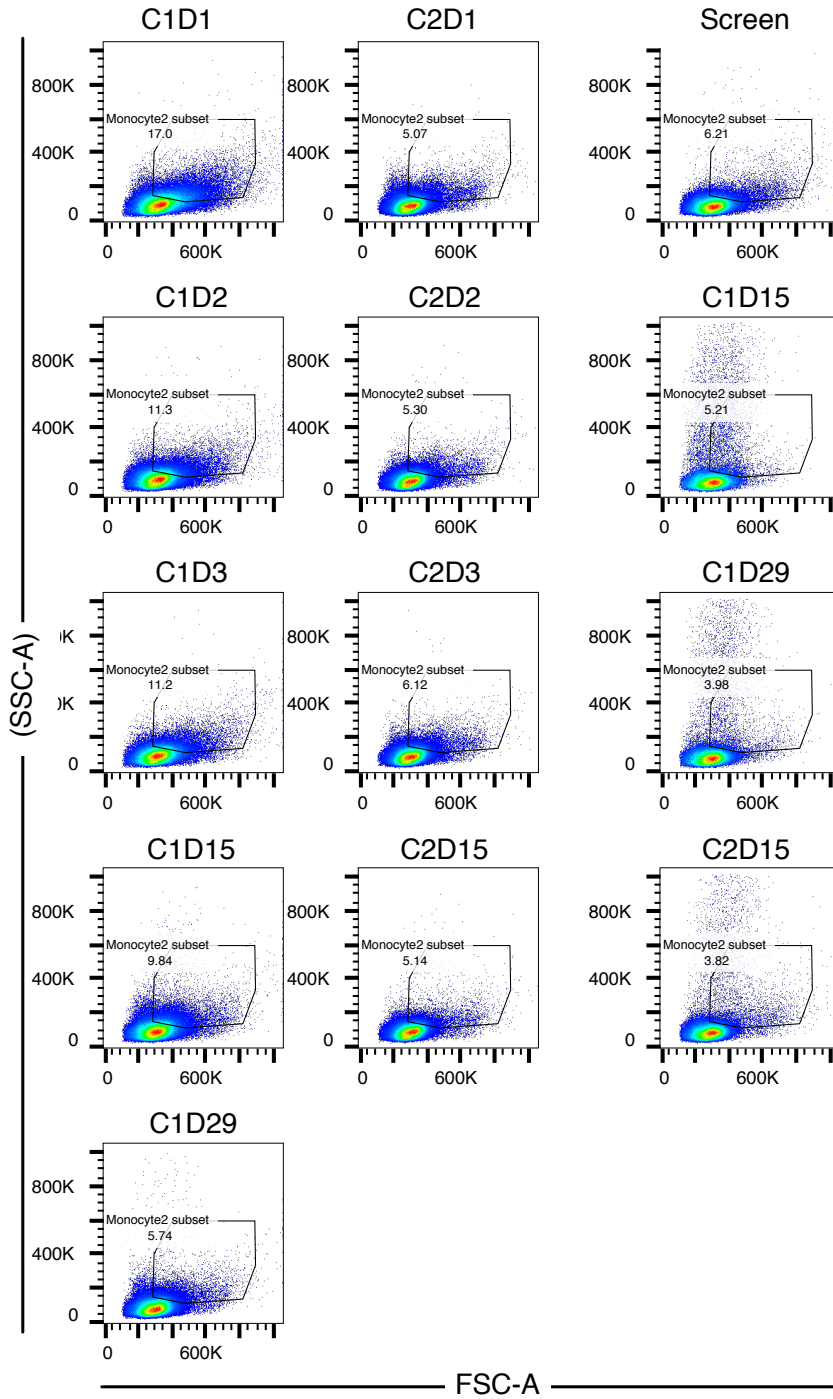
Bone Marrow



MDSC4 Base Gate (CD45+FSCintSSCint)

Blood

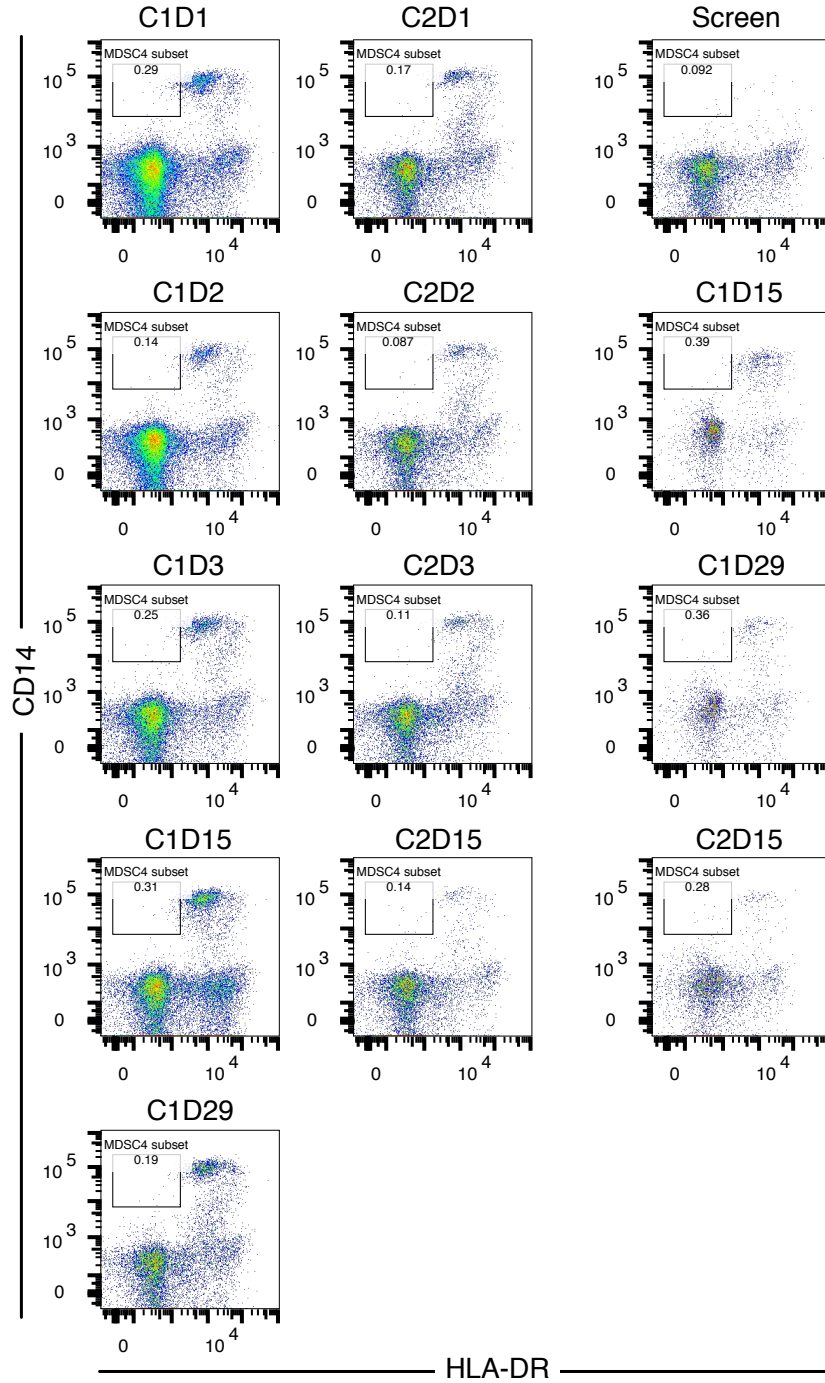
Bone Marrow



MDSC4 (CD45+CD14+HLADRlo/-)

Blood

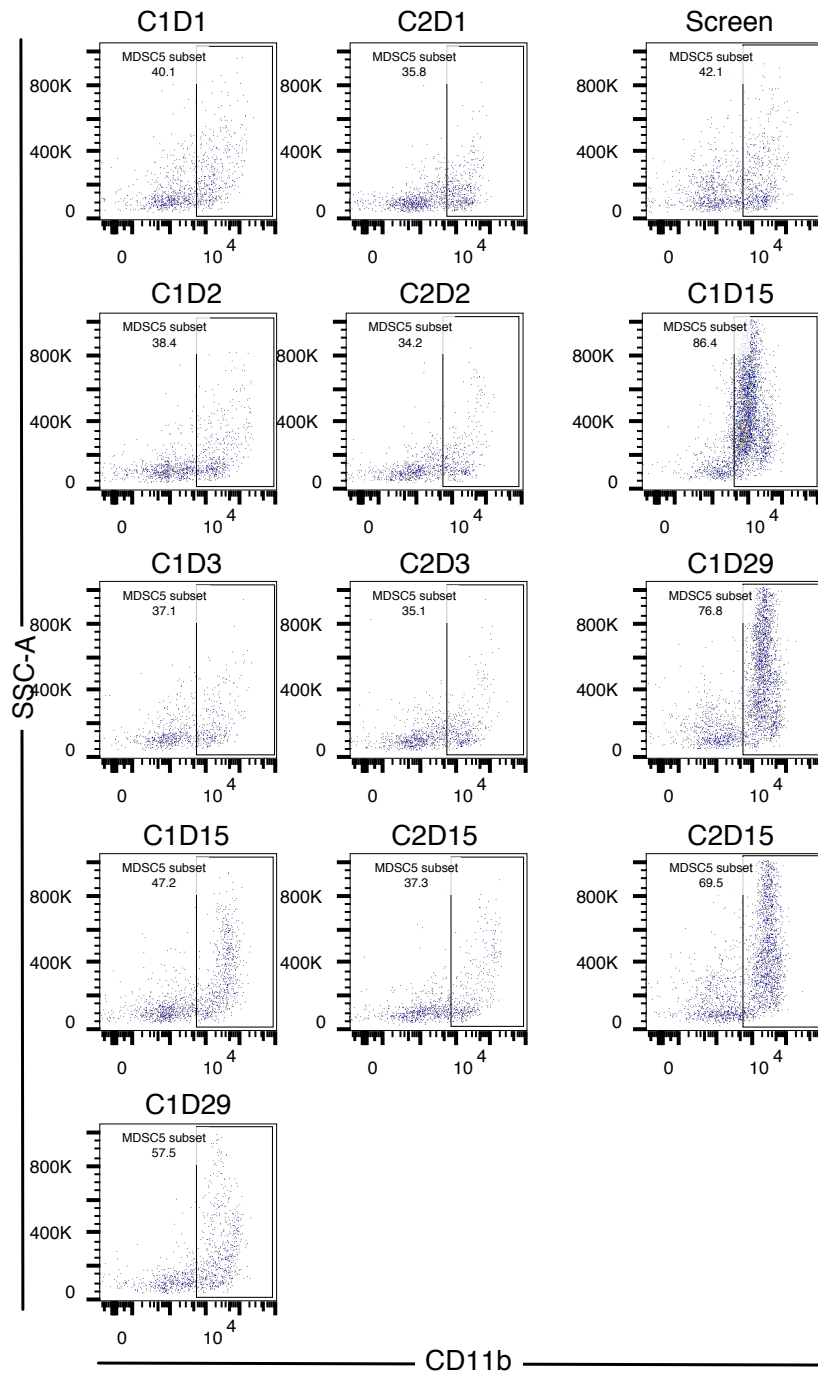
Bone Marrow



MDSC5 (CD45+CD15+CD14-CD11b+)

Blood

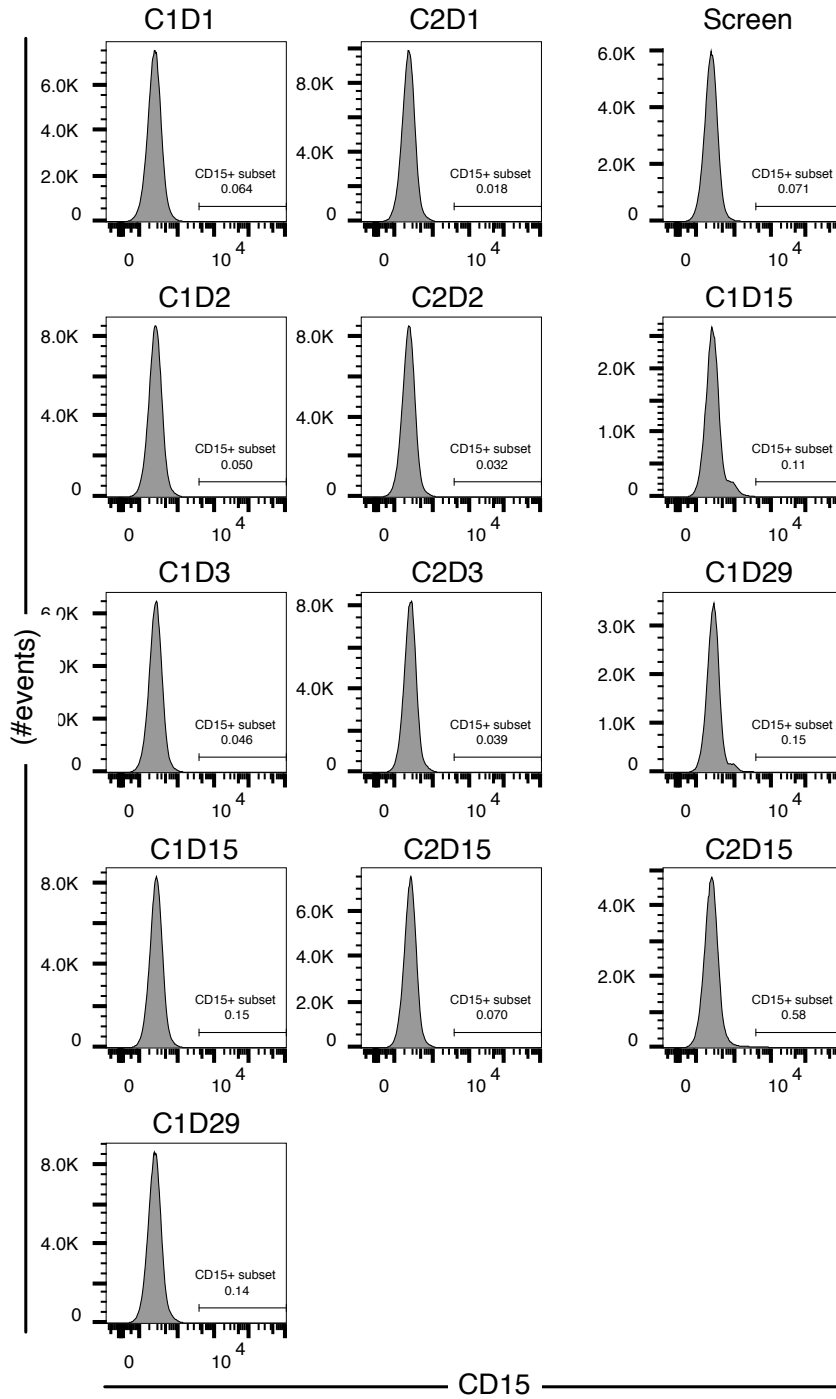
Bone Marrow



MDSC6 Base Gate (CD45+CD15hi)

Blood

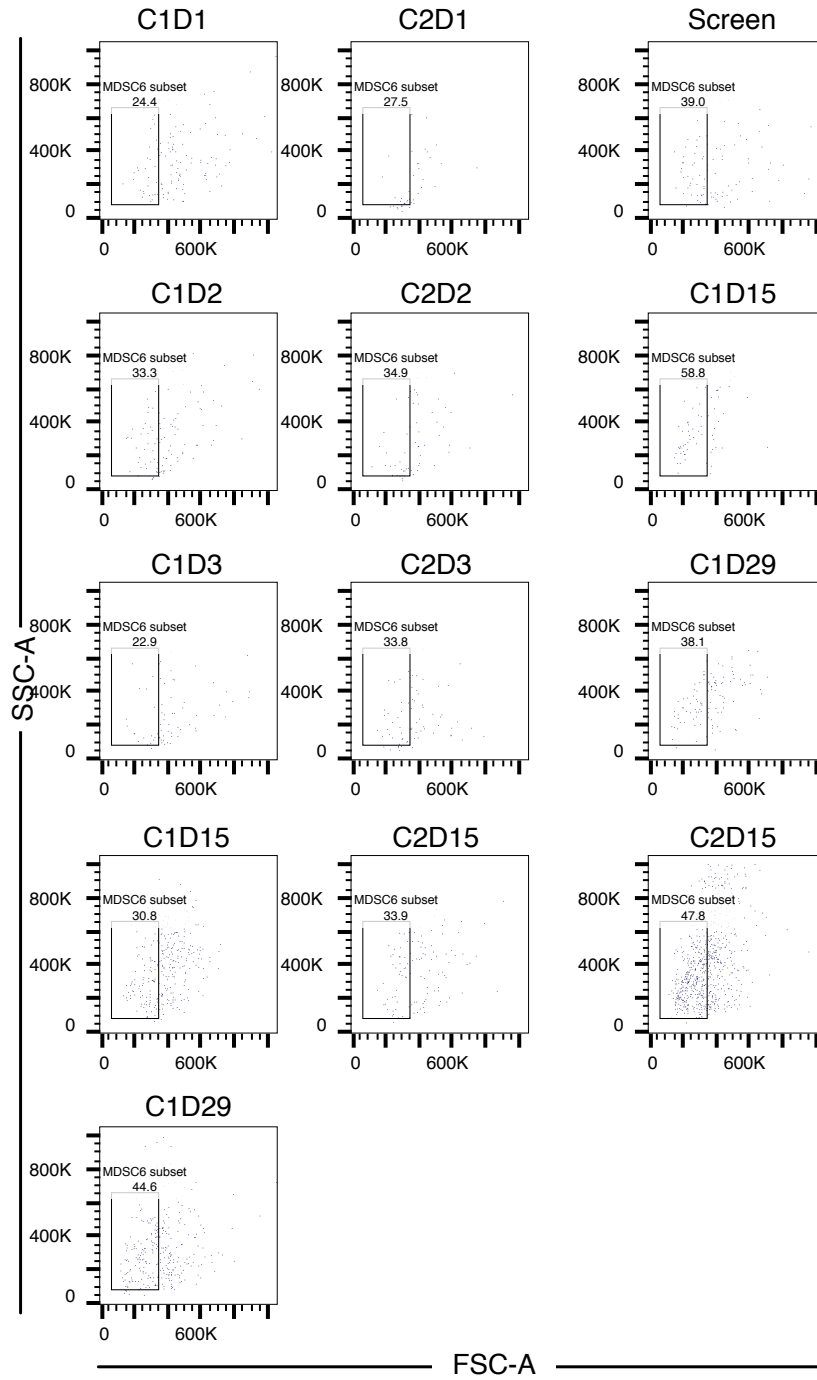
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MDSC6 (CD45+CD15hiSSChiFSClo)

Blood

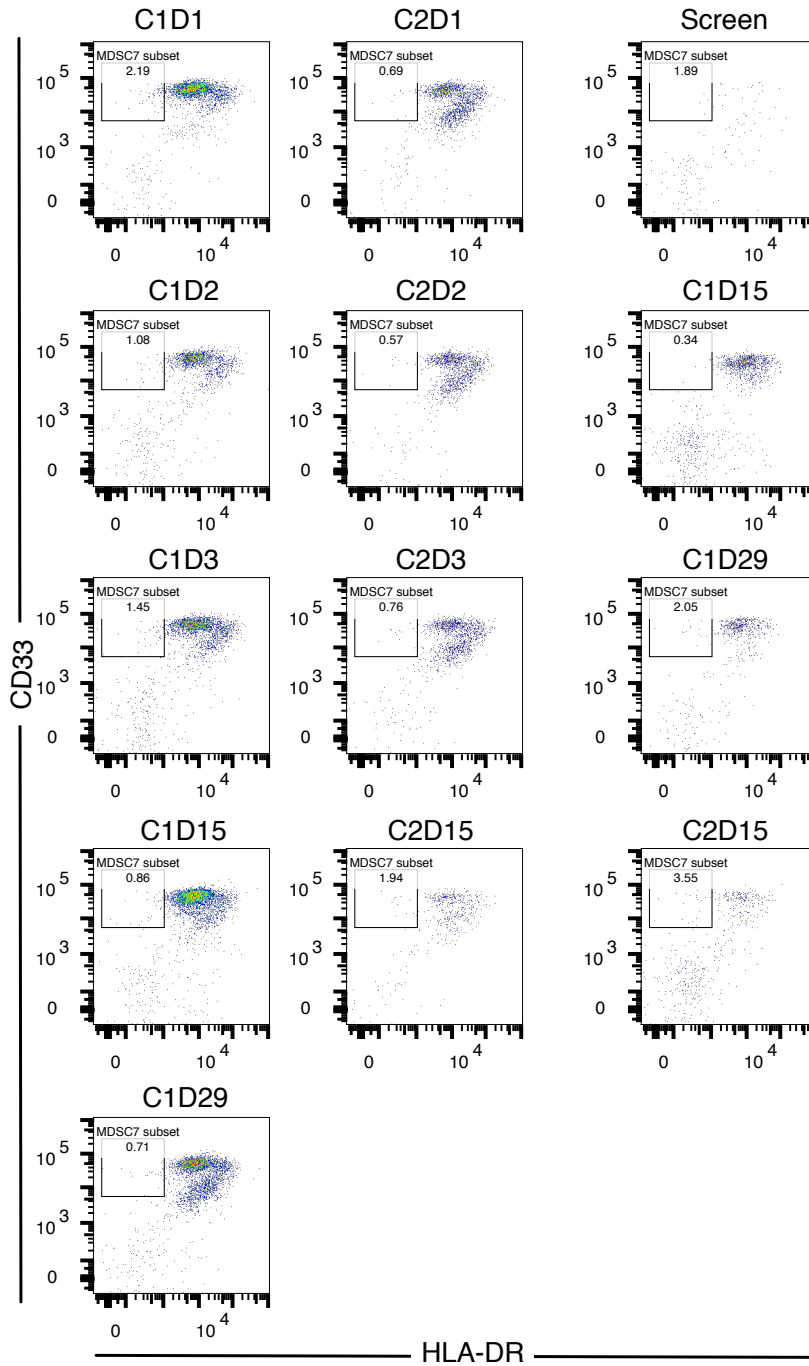
Bone Marrow



MDSC7 (CD45+CD15-CD14+CD33hiHLADRIo)

Blood

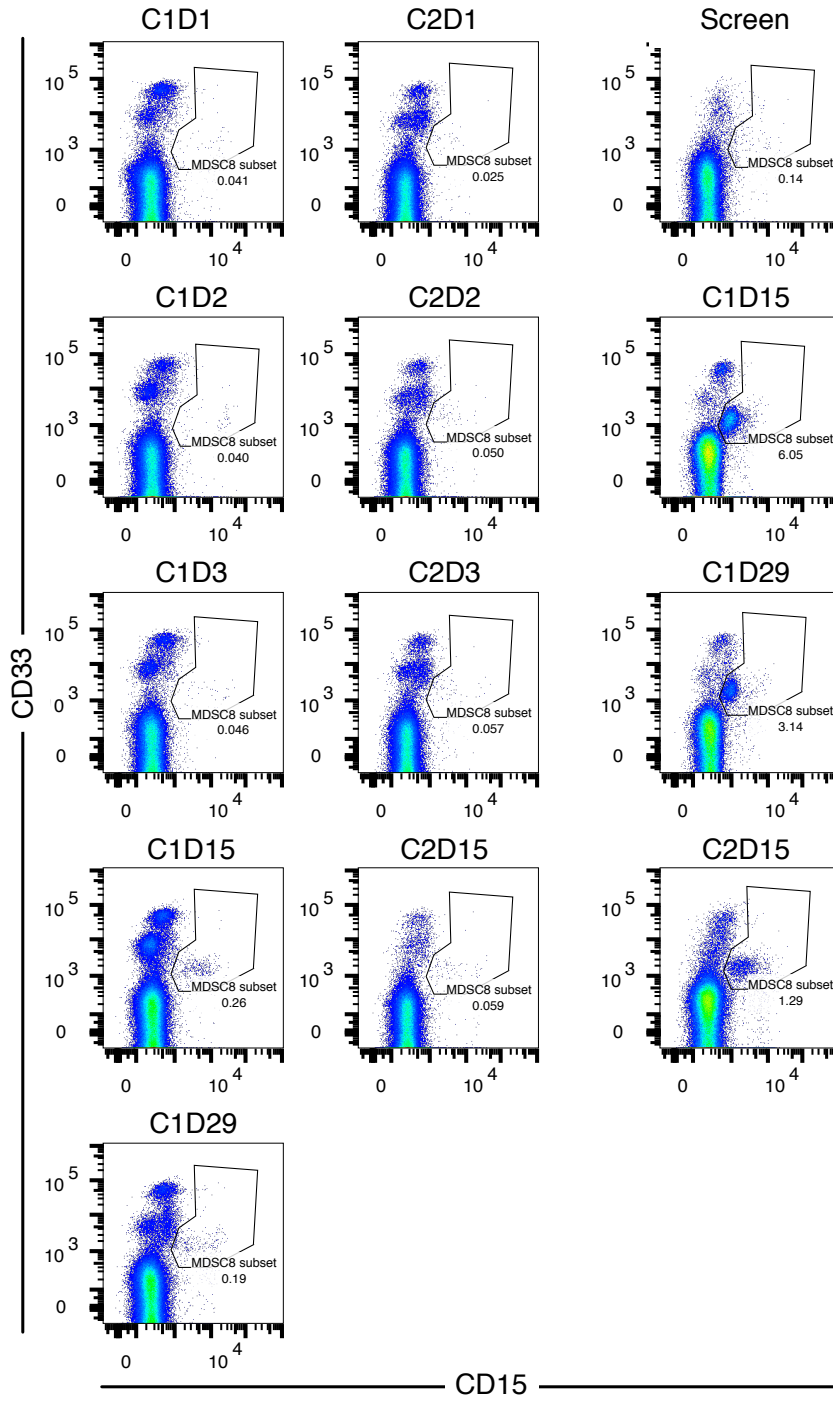
Bone Marrow



MDSC8 (CD45+CD15+CD33+)

Blood

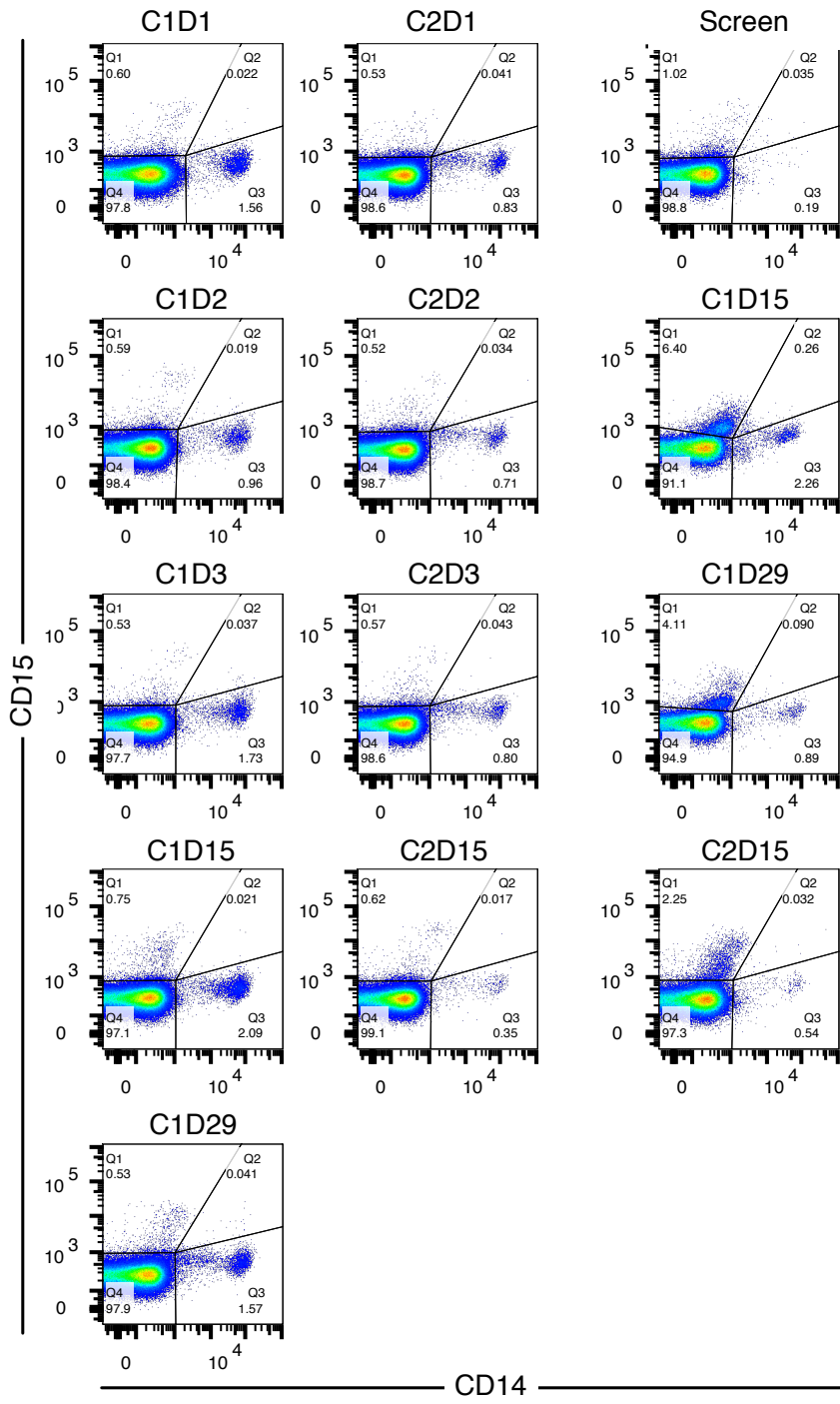
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MDSC5/7/9 Base Gate (CD45+)

Blood

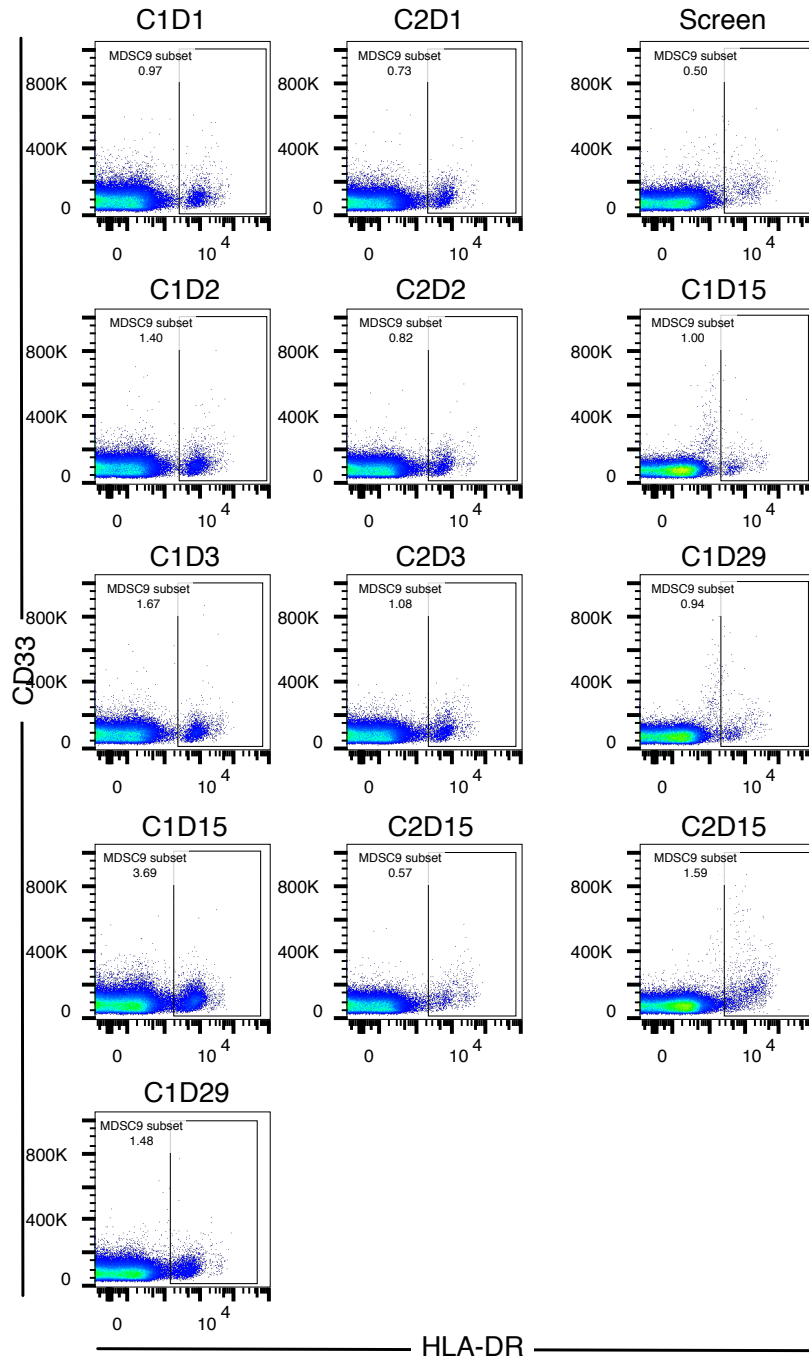
Bone Marrow



MDSC9 (CD45+CD15-CD14-CD33hi)

Blood

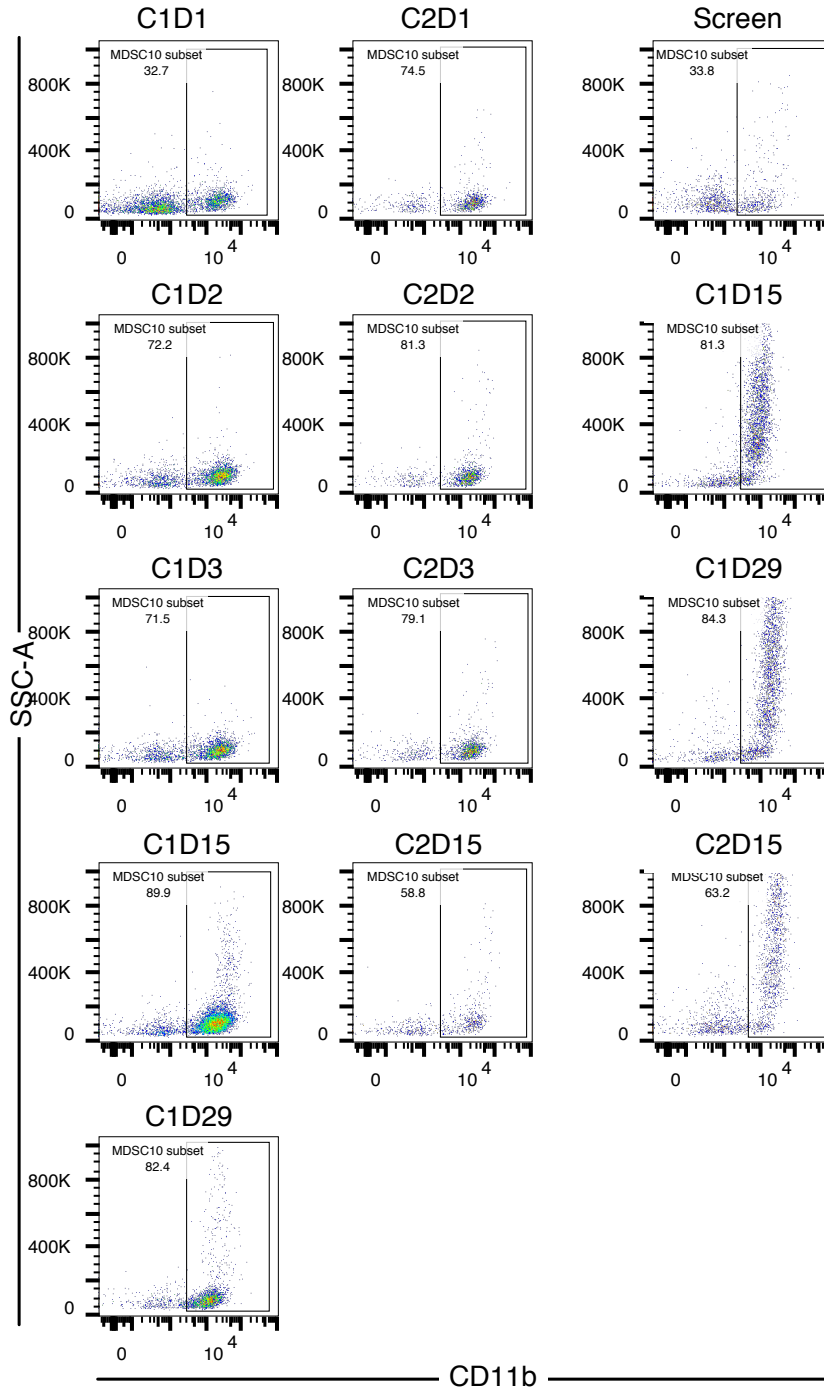
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MDSC10 (CD45+Lin-HLADR-CD11b+)

Blood

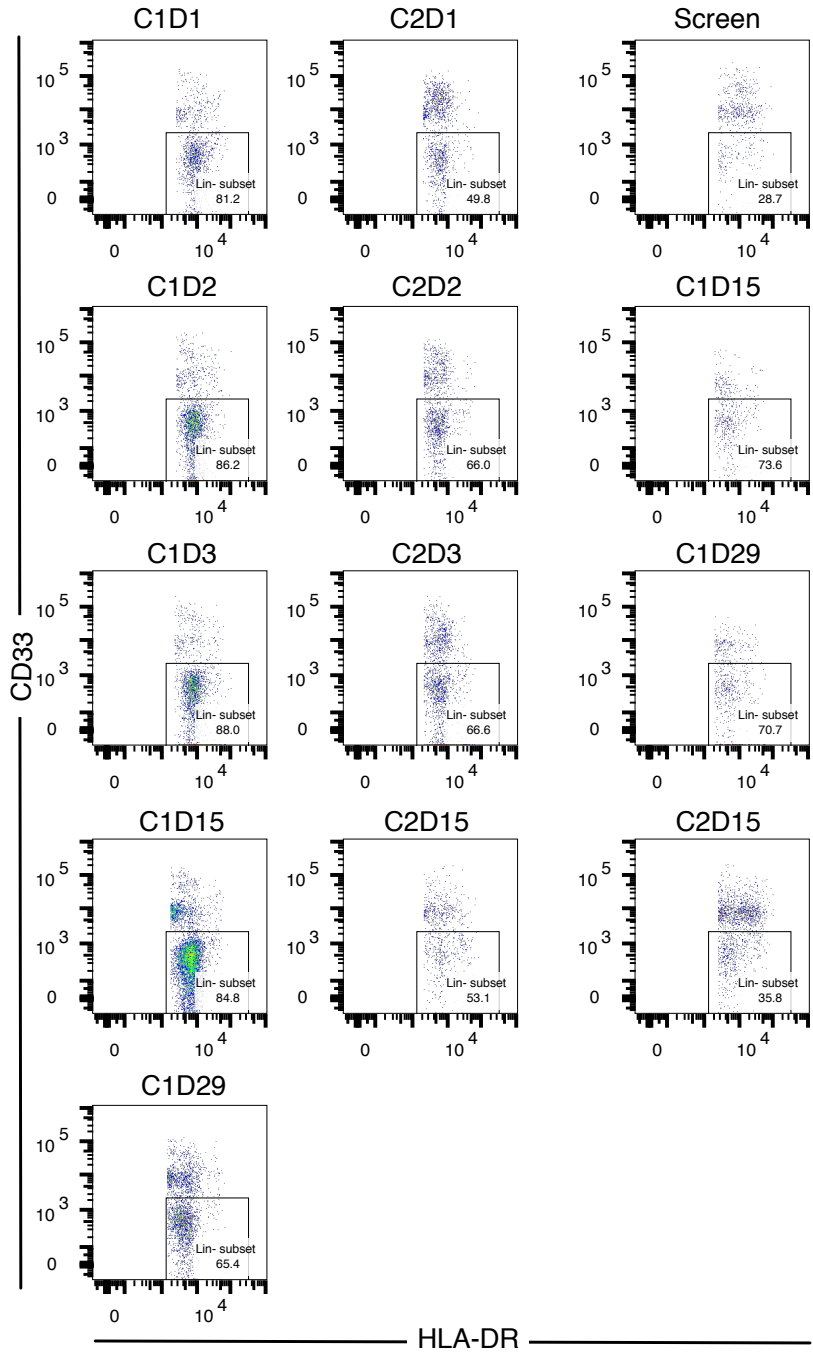
Bone Marrow



Moffitt MDSC (CD45+CD15-CD14-Lin-CD33hi)

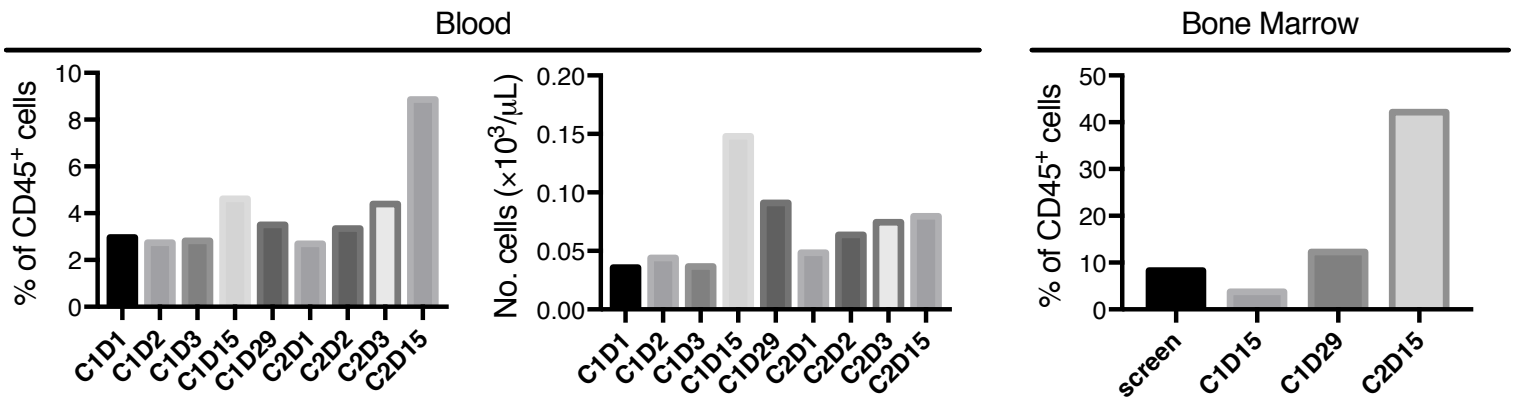
Blood

Bone Marrow

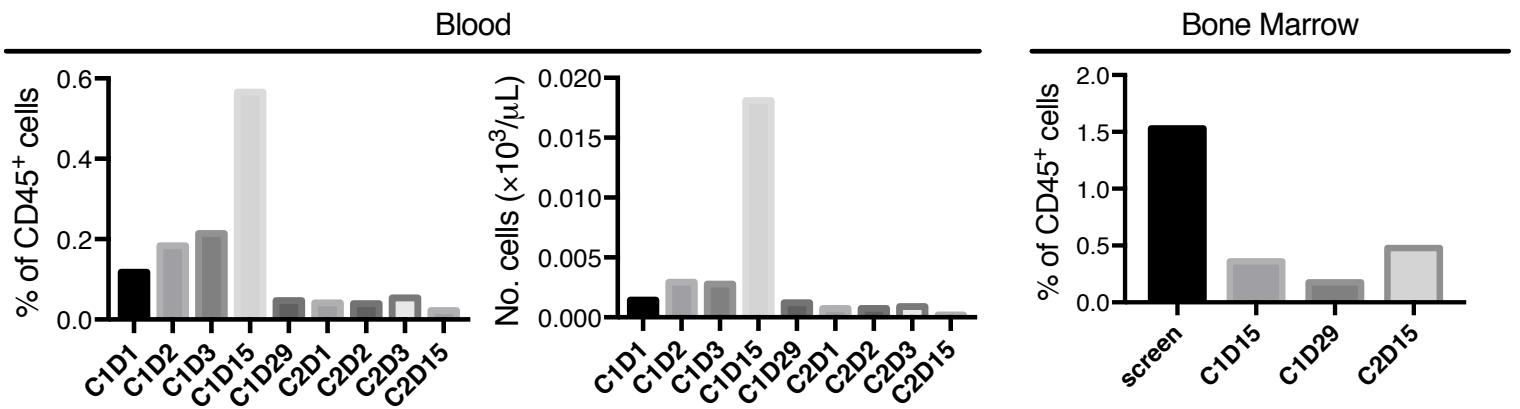


**Analysis of AML blasts
mAb Set #2**

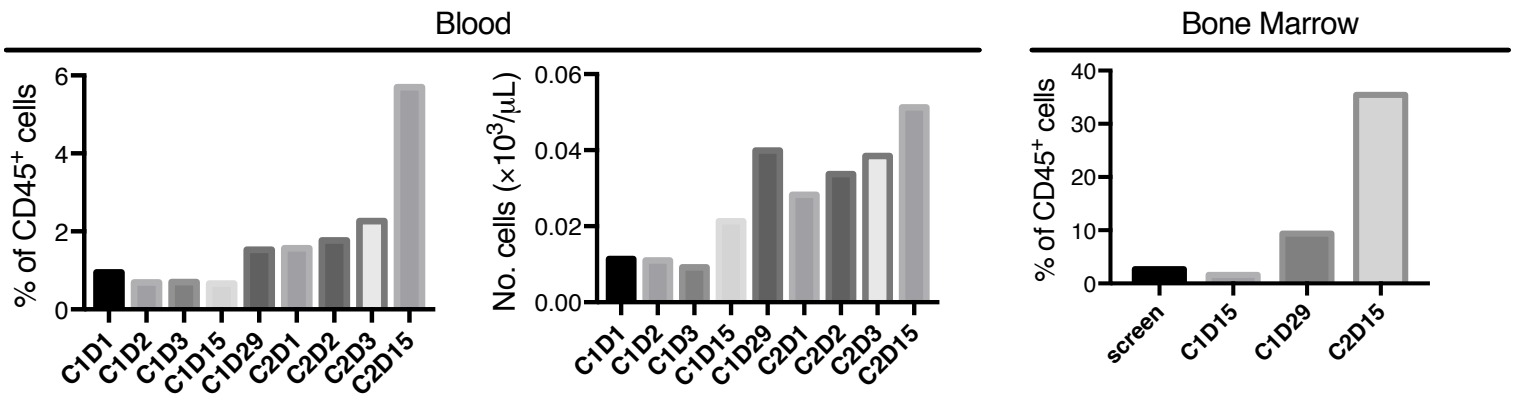
Total AML Blasts



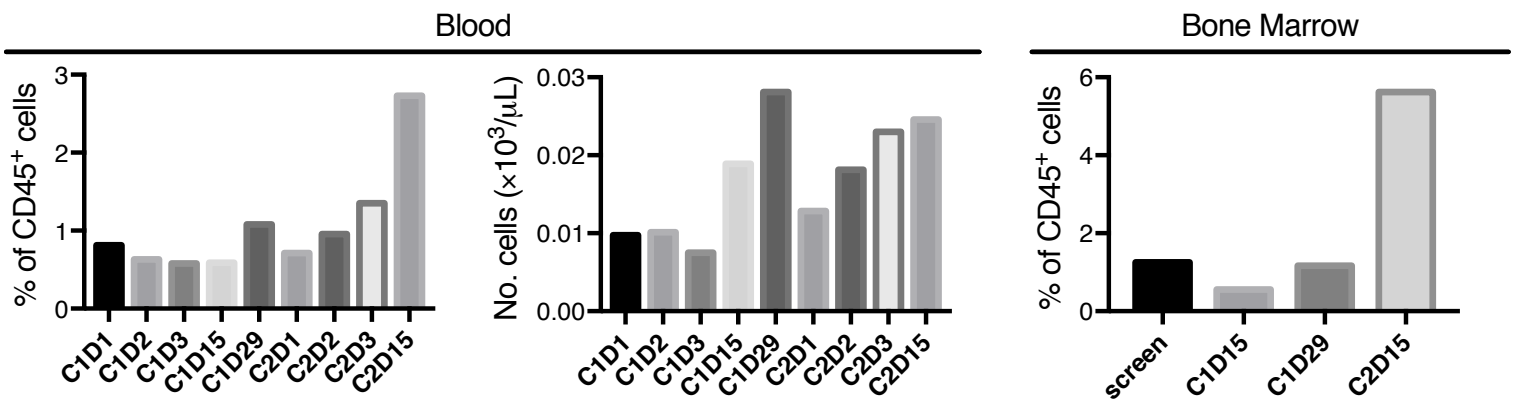
CD117⁺CD34⁻ AML Blasts



CD117⁺CD34⁺ AML Blasts



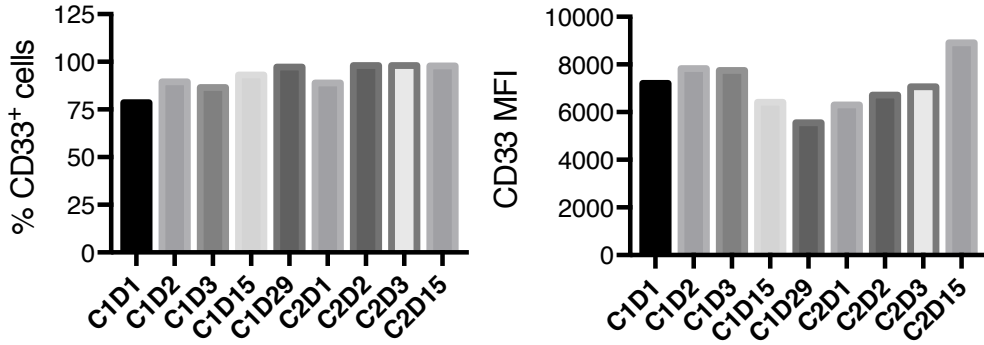
CD117⁻CD34⁺ AML Blasts



Expression of CD33 on AML Blasts mAb Set #2

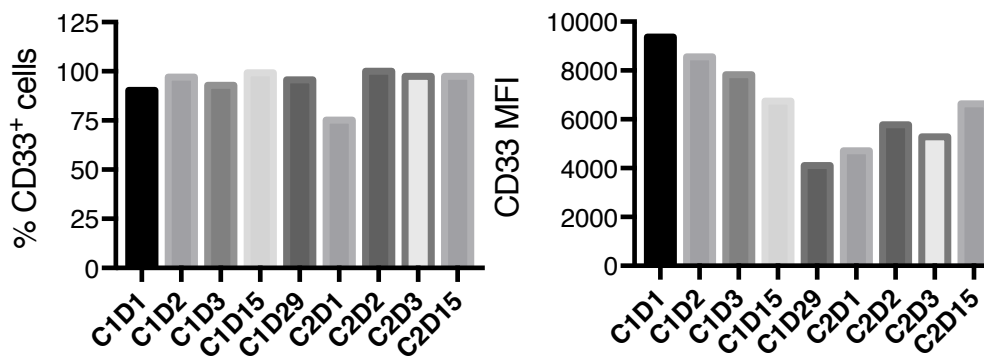
Total AML Blasts

Blood



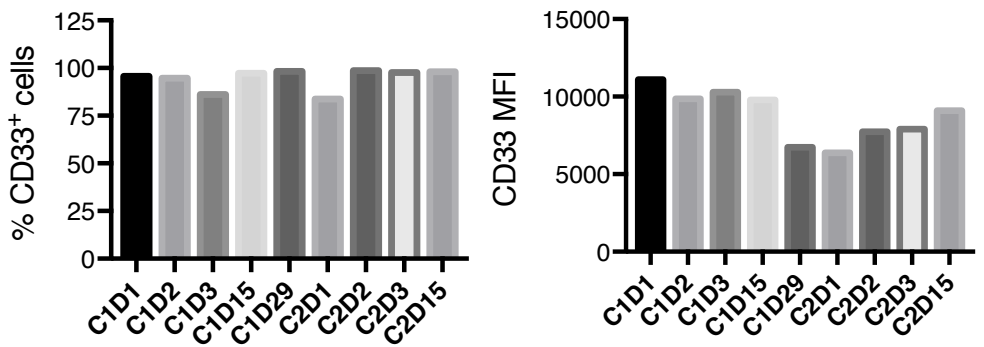
CD117⁺CD34⁻ AML Blasts

Blood



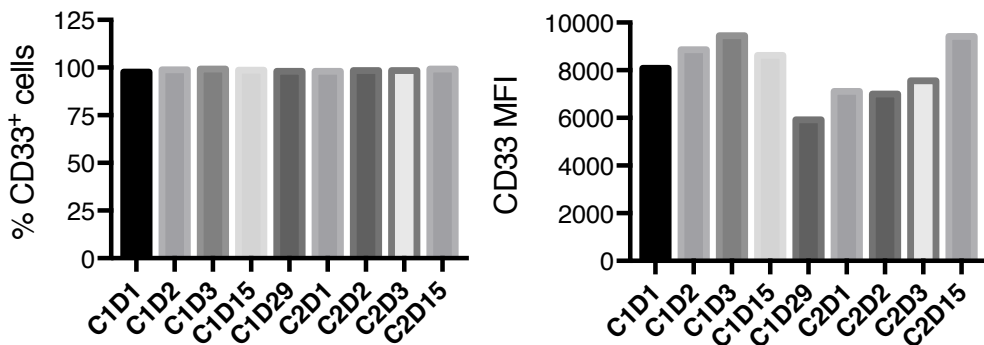
CD117⁺CD34⁺ AML Blasts

Blood



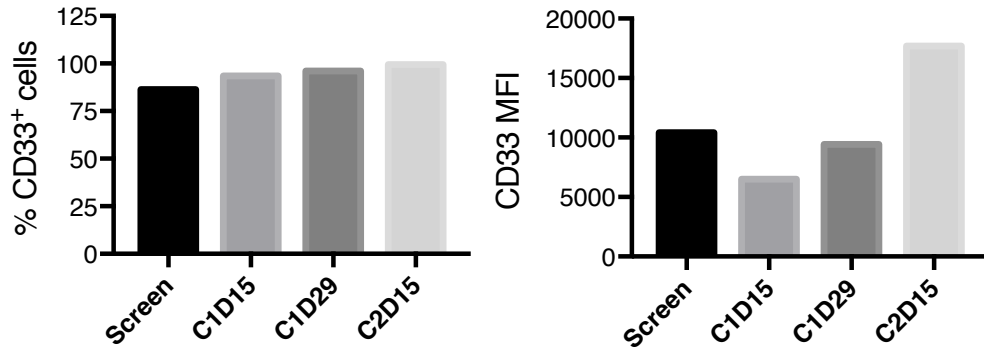
CD117⁻CD34⁺ AML Blasts

Blood



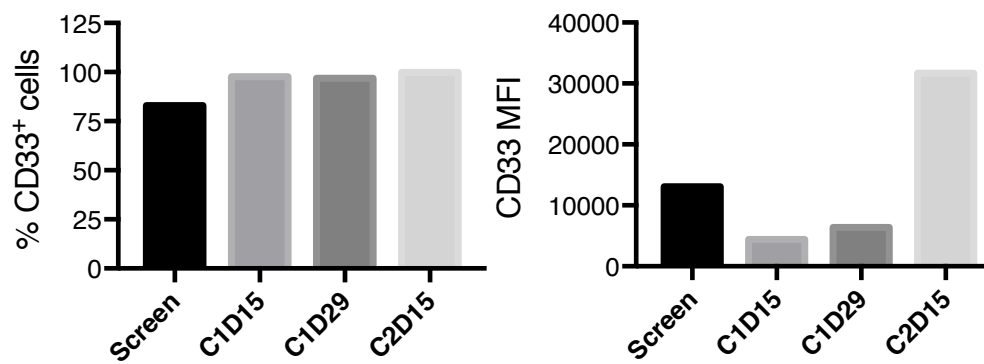
Total AML Blasts

Bone Marrow



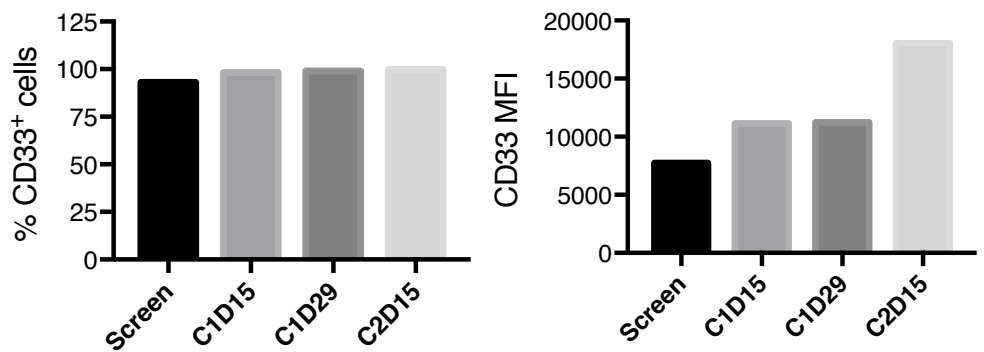
CD117⁺CD34⁻ AML Blasts

Bone Marrow



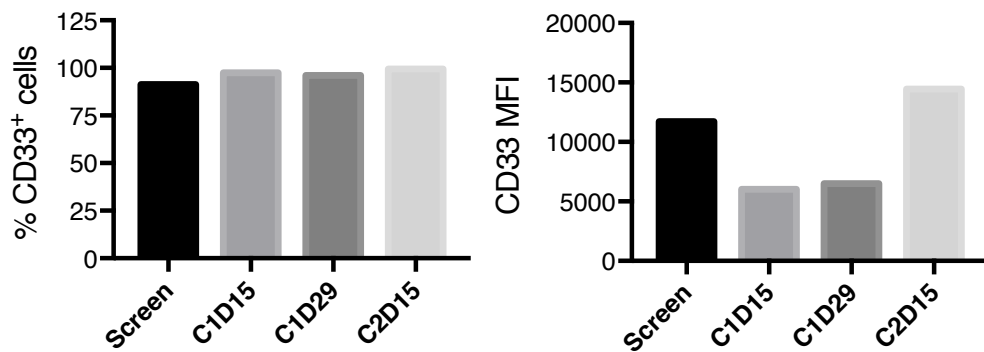
CD117⁺CD34⁺ AML Blasts

Bone Marrow



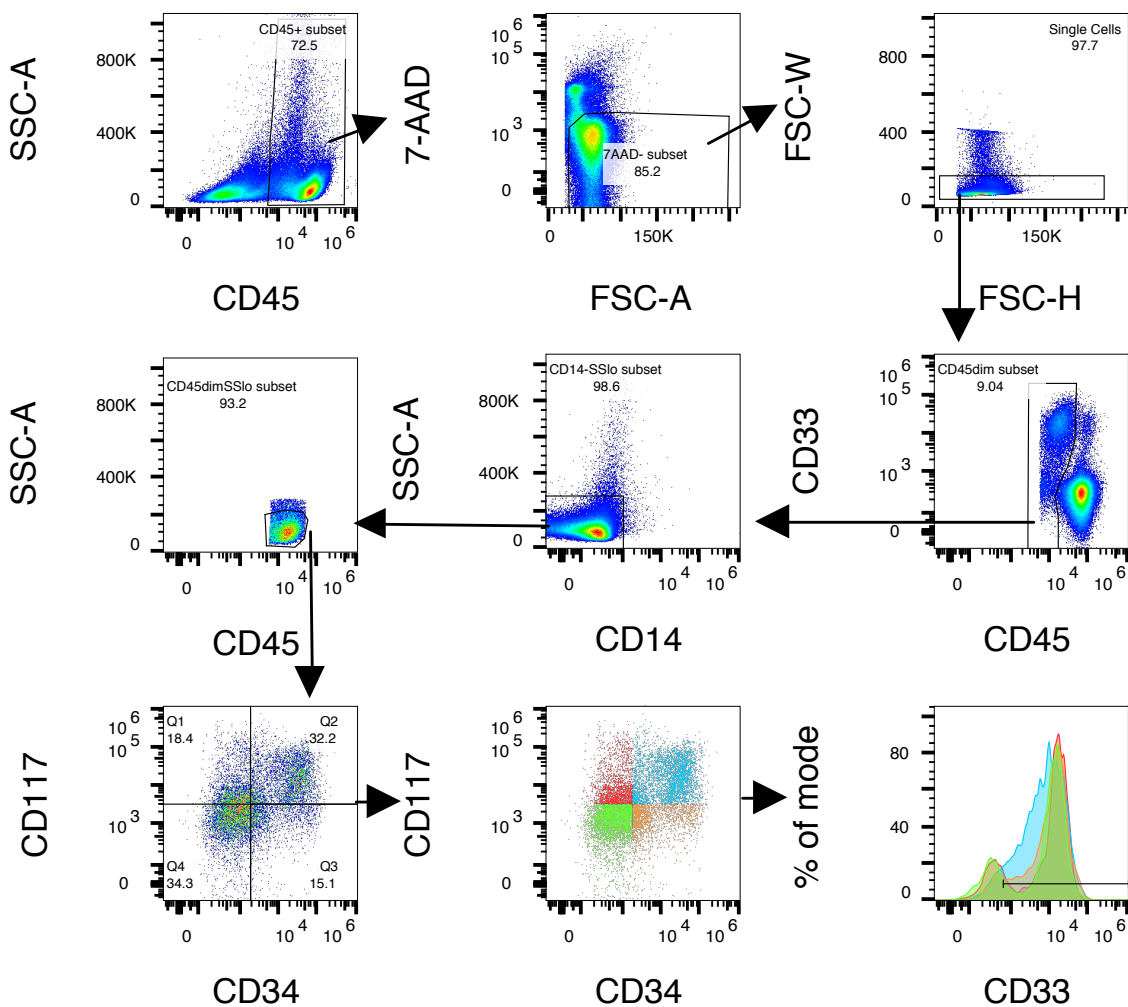
CD117⁻CD34⁺ AML Blasts

Bone Marrow

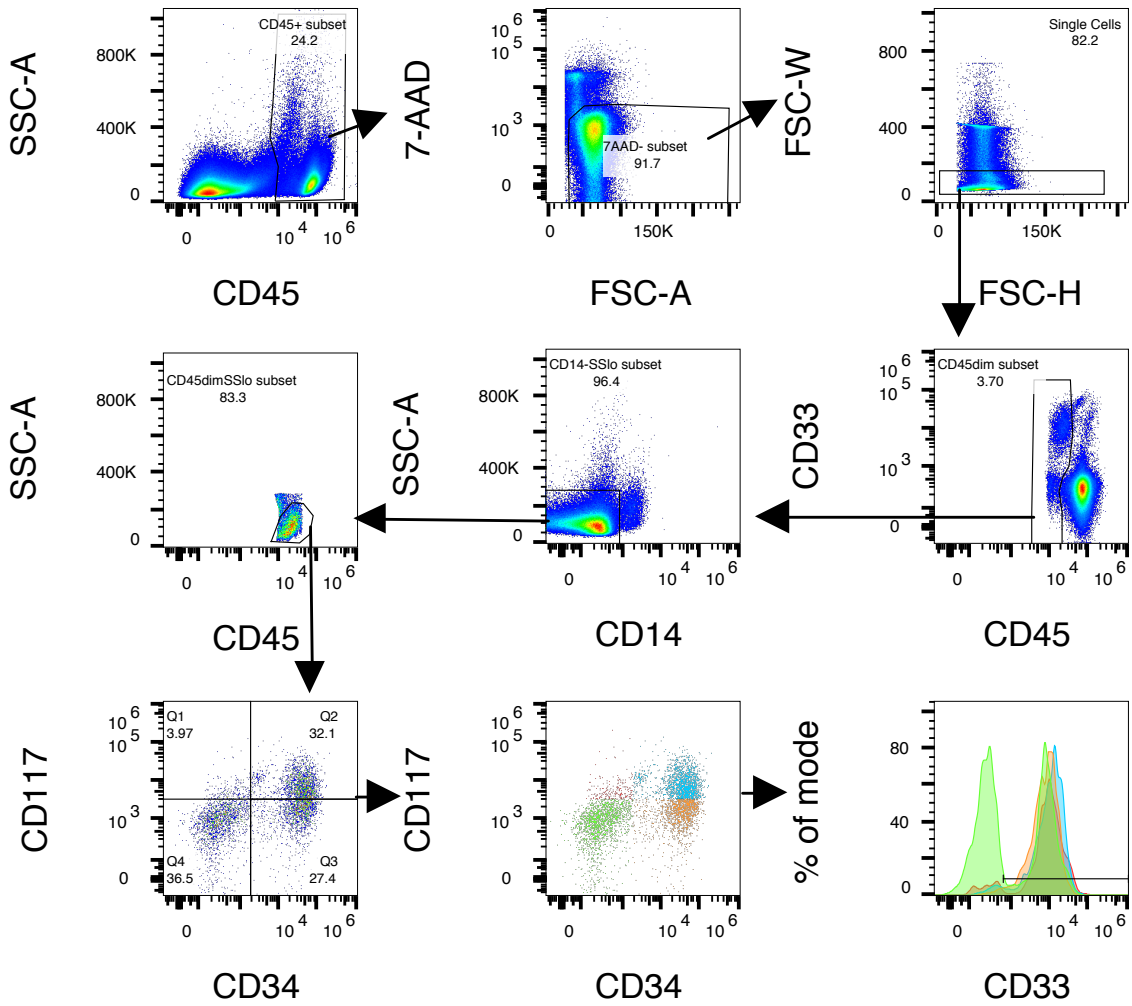


**FACS profiles of AML Blasts
mAb Set #2**

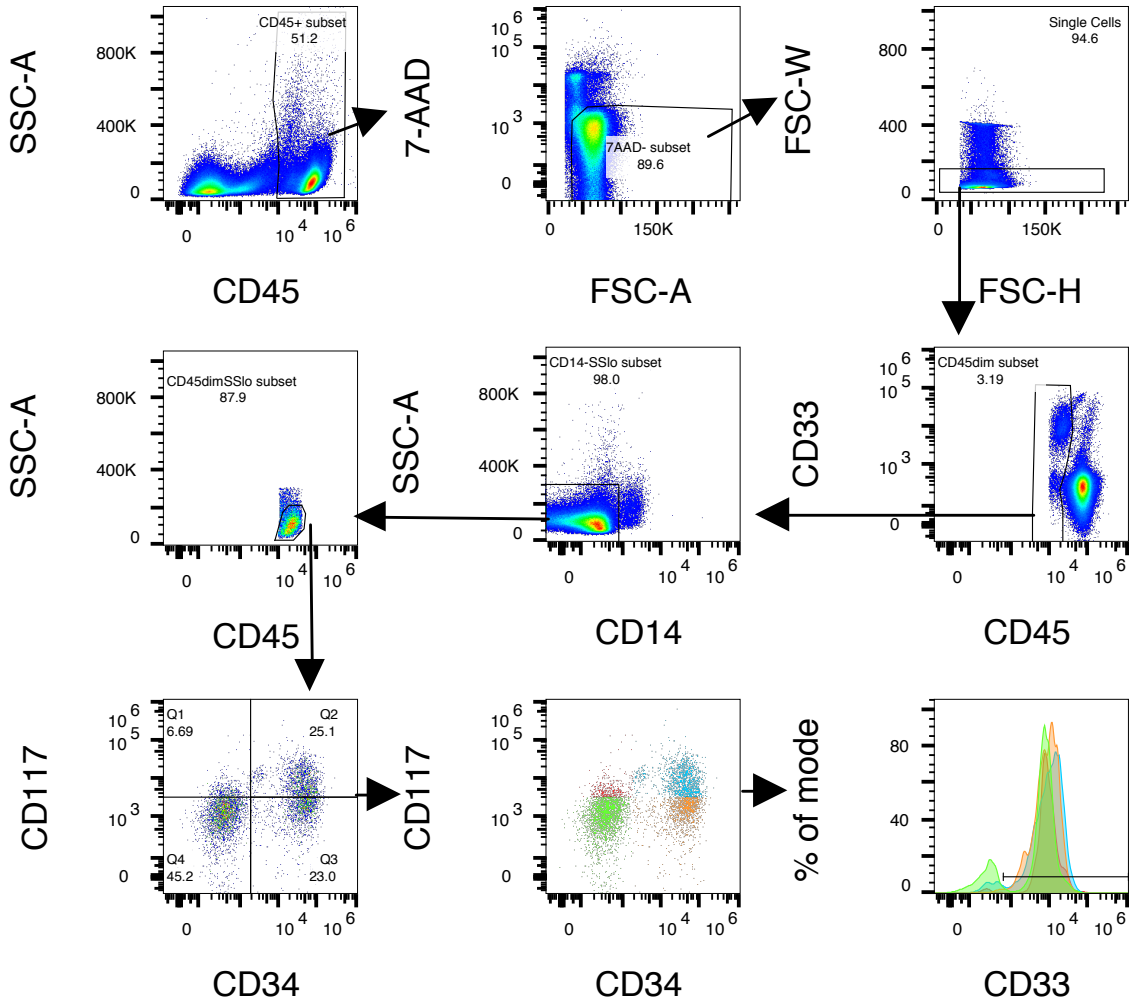
Patient 002-004 BM Screen



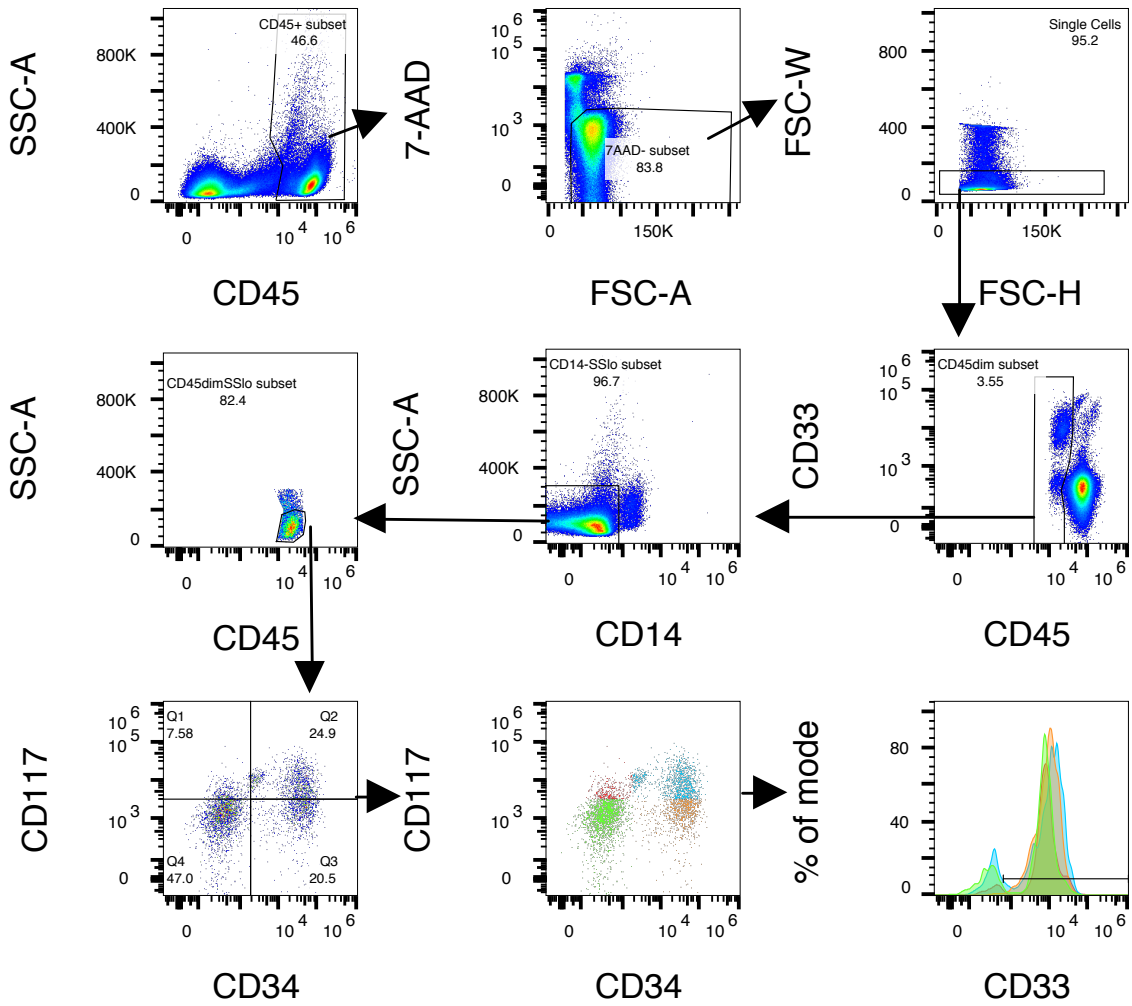
Patient 002-004 Blood C1D1



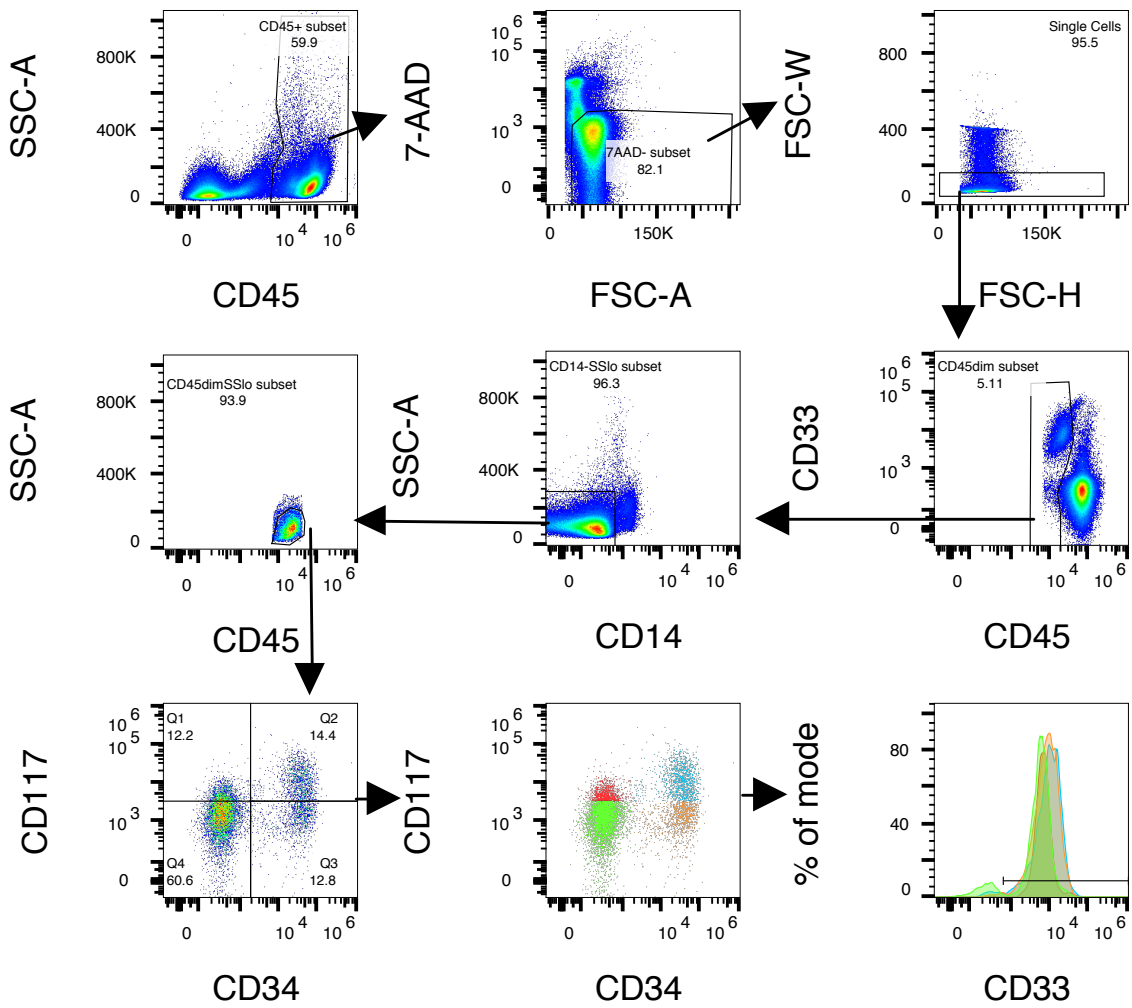
Patient 002-004 Blood C1D2



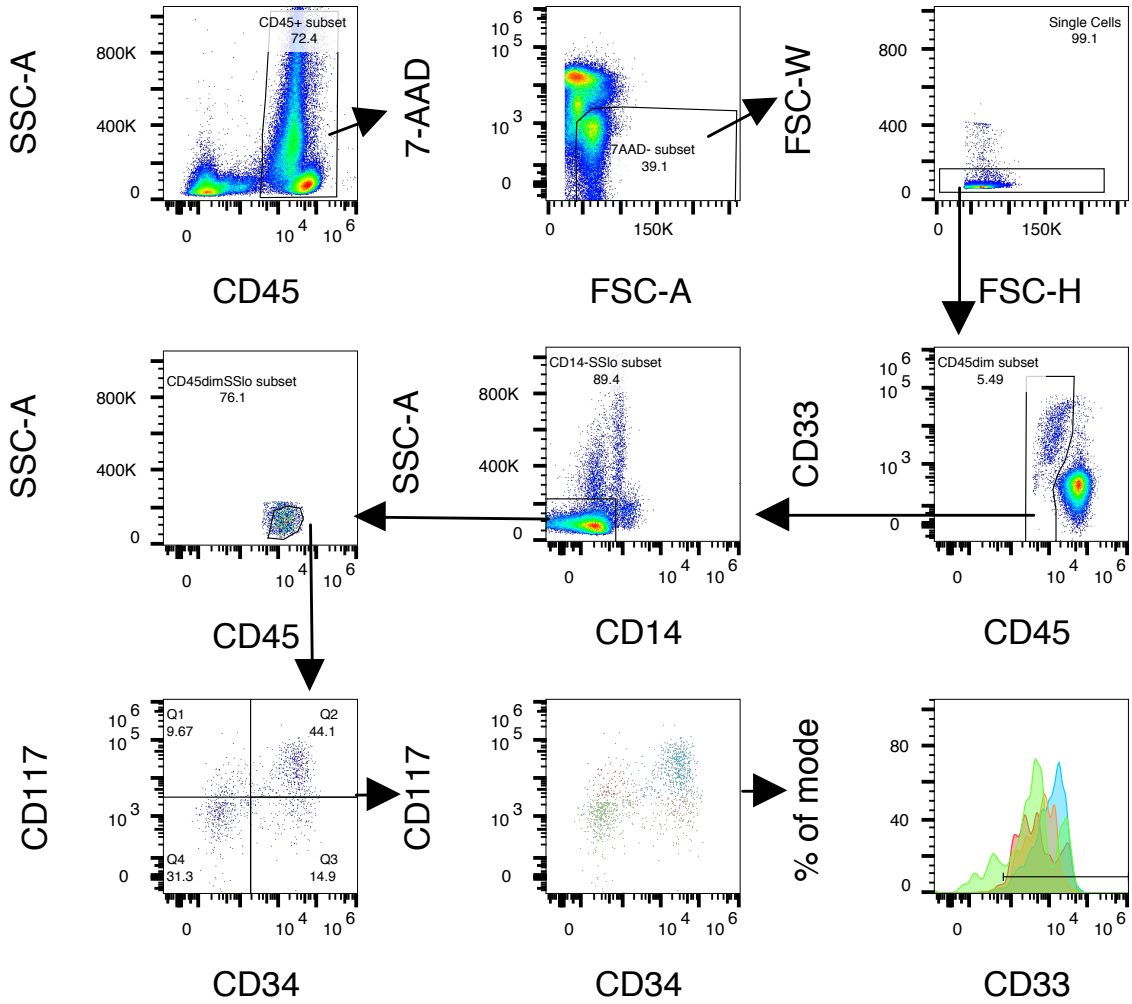
Patient 002-004 Blood C1D3



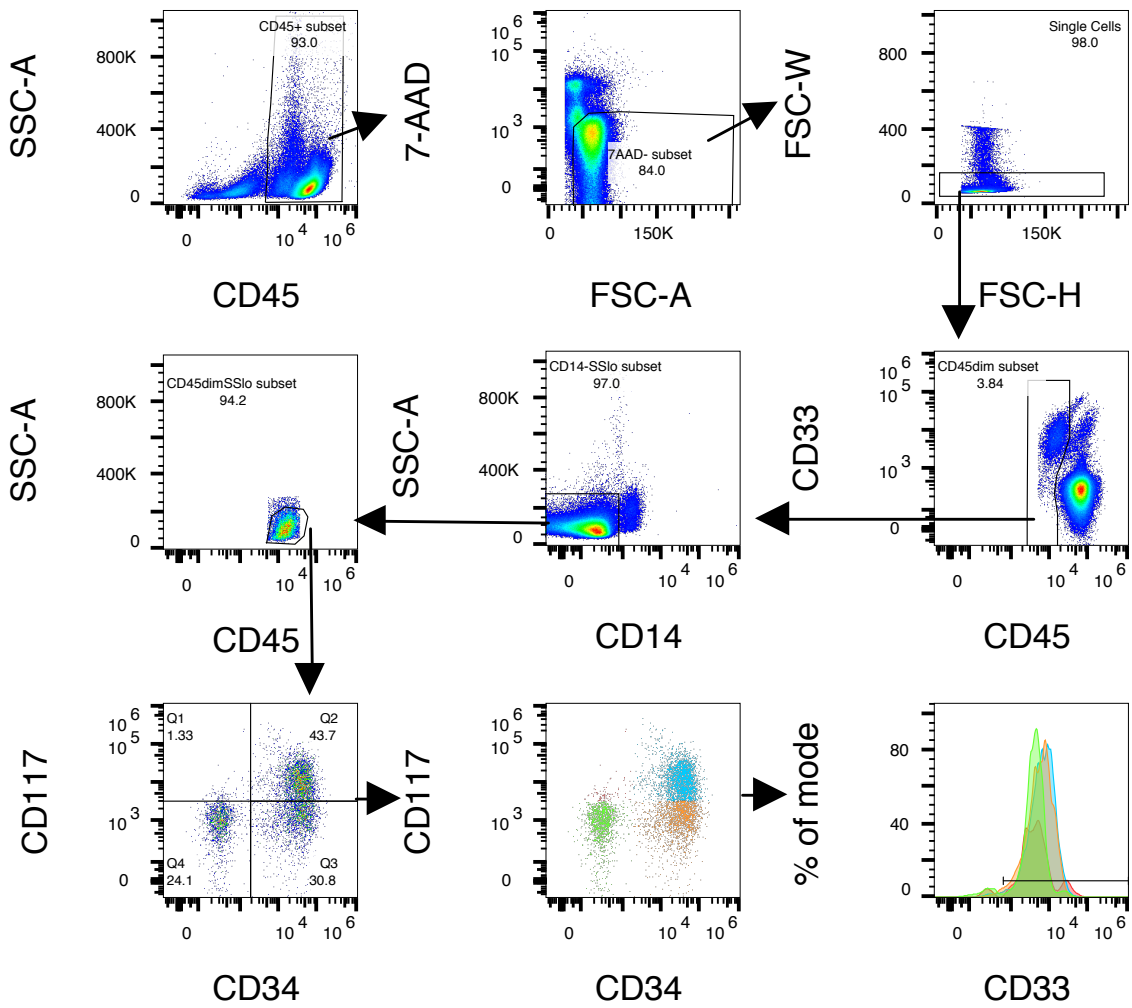
Patient 002-004 Blood C1D15



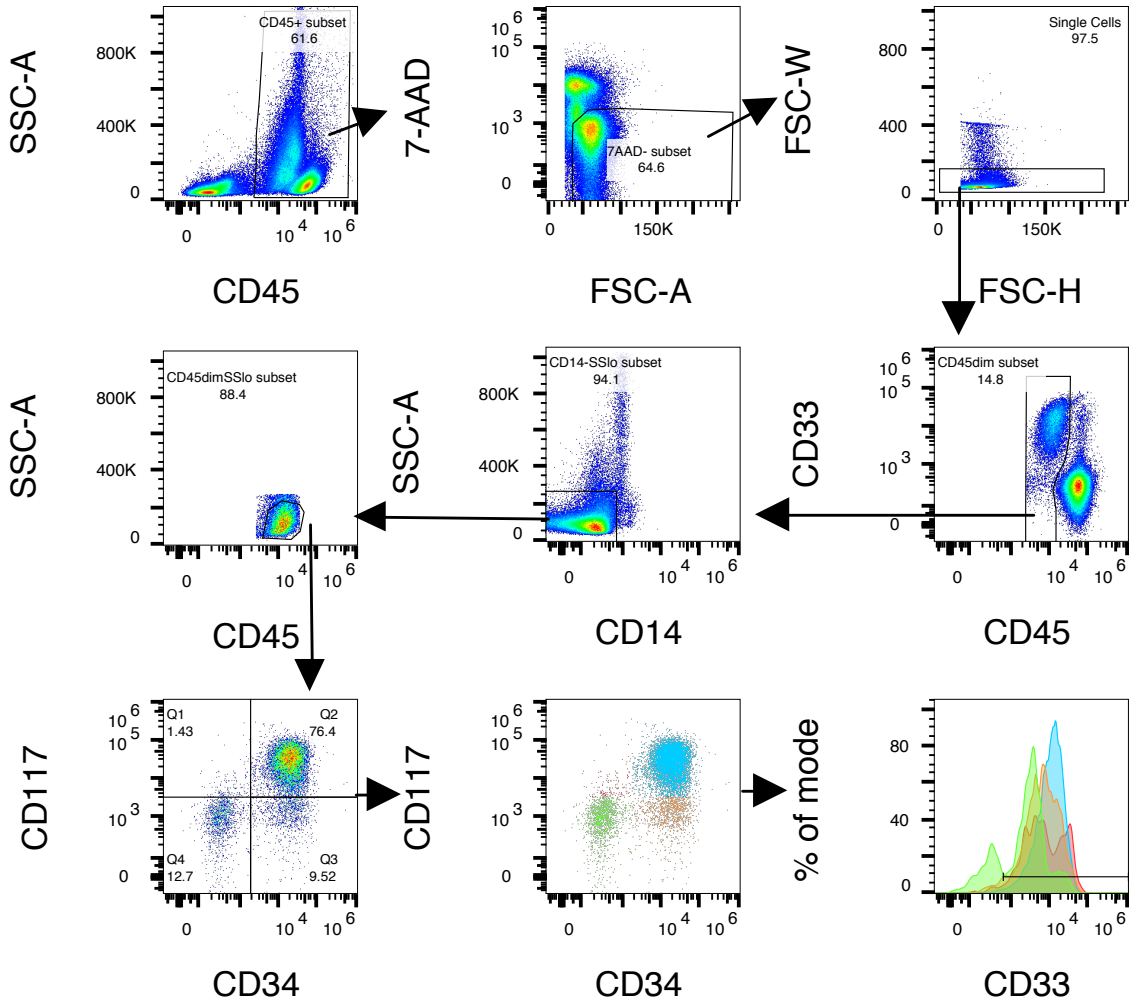
Patient 002-004 BM C1D15



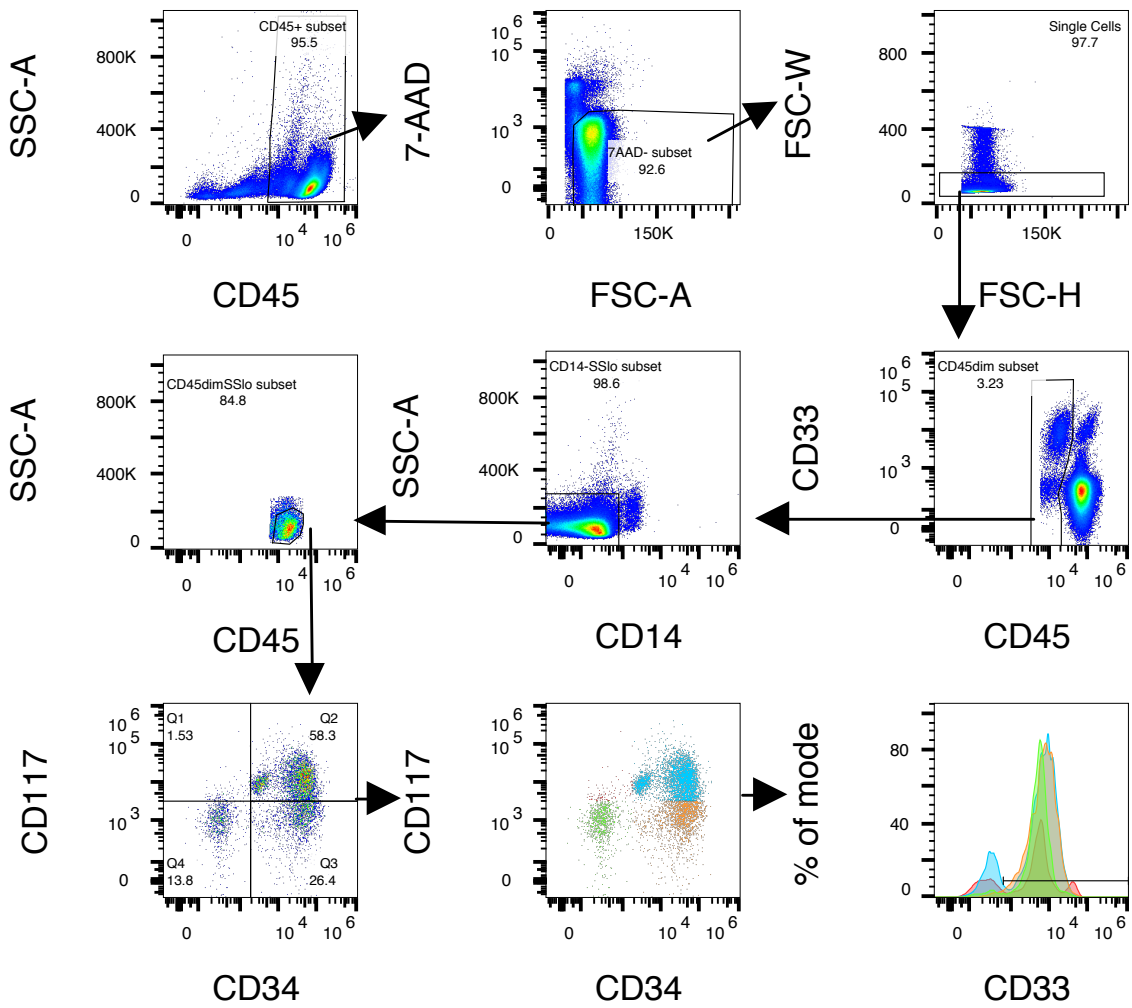
Patient 002-004 Blood C1D29



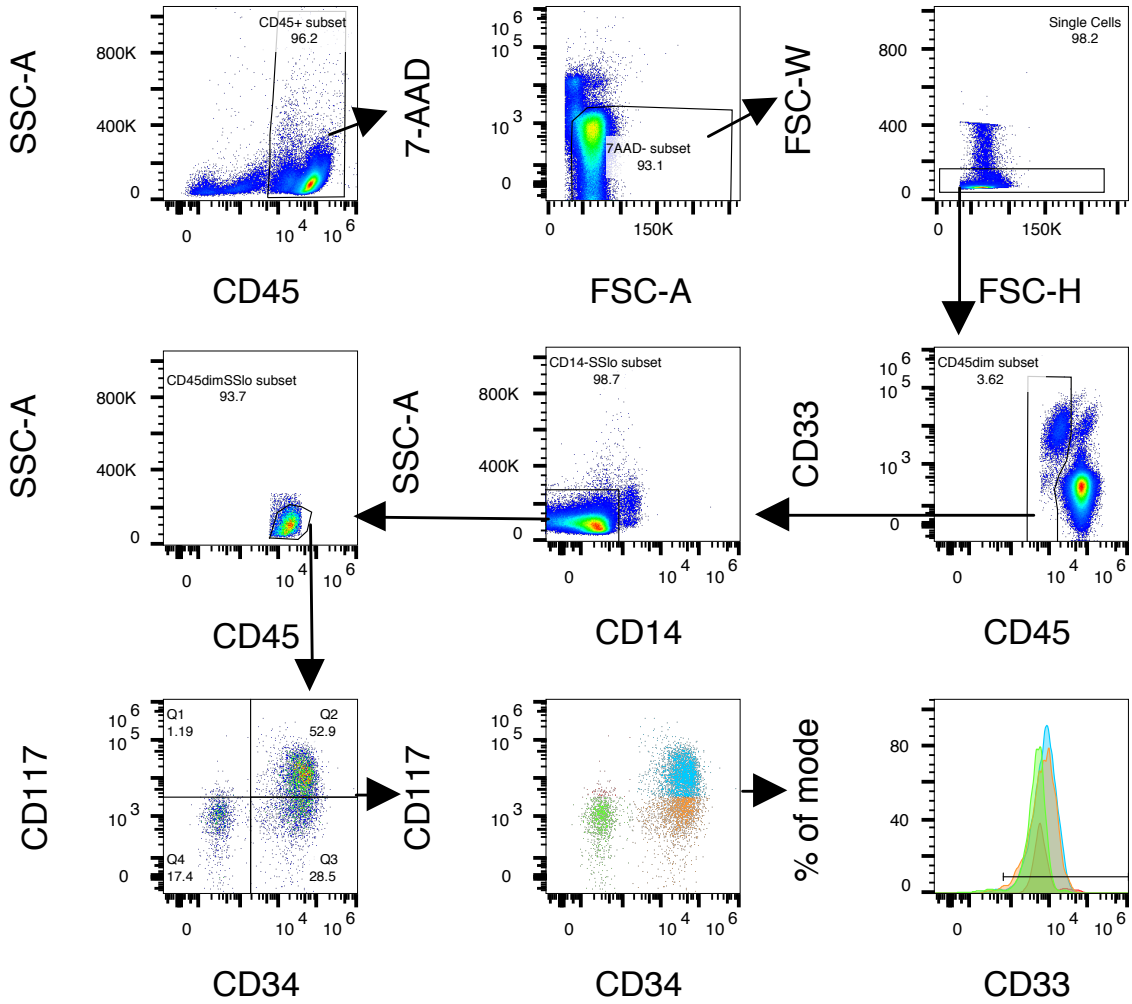
Patient 002-004 BM C1D29



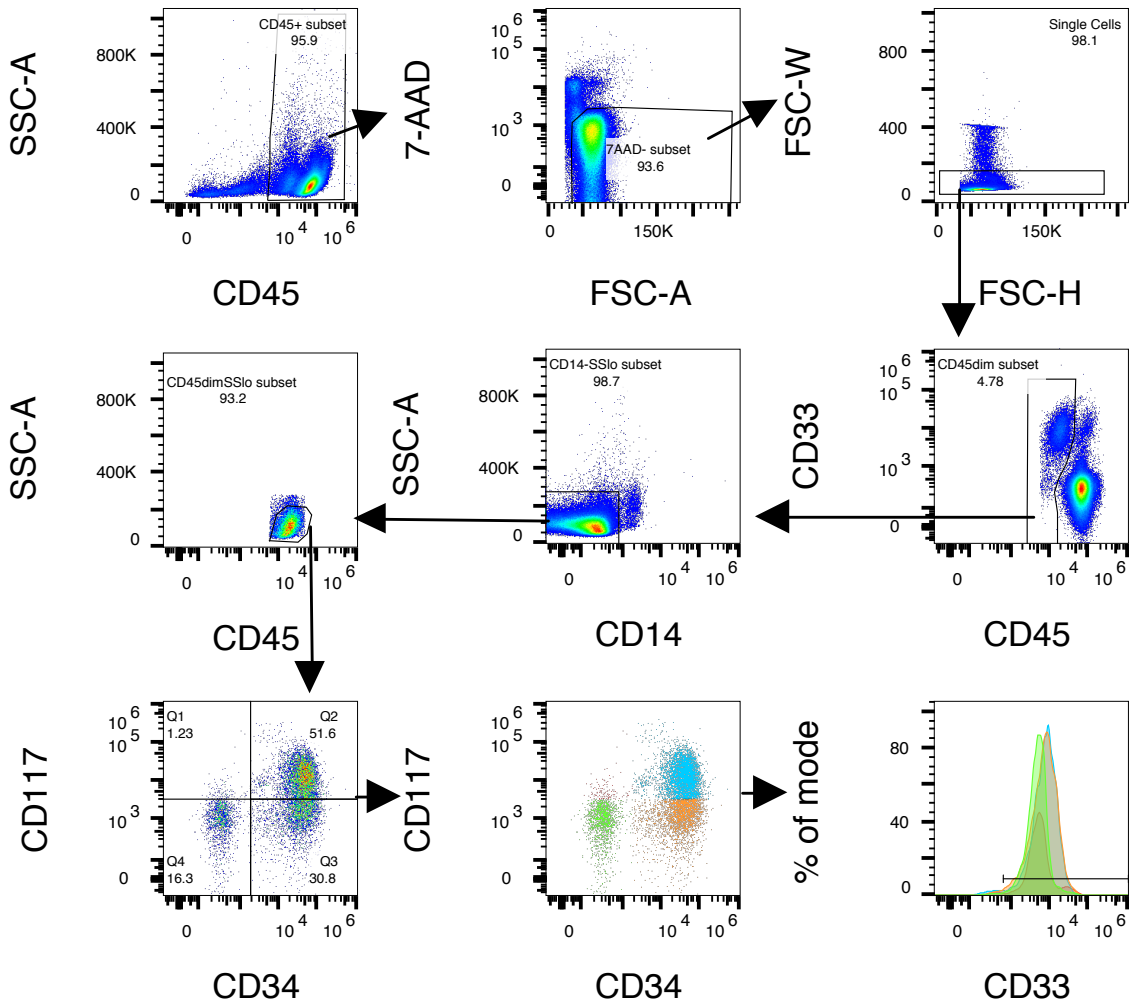
Patient 002-004 Blood C2D1



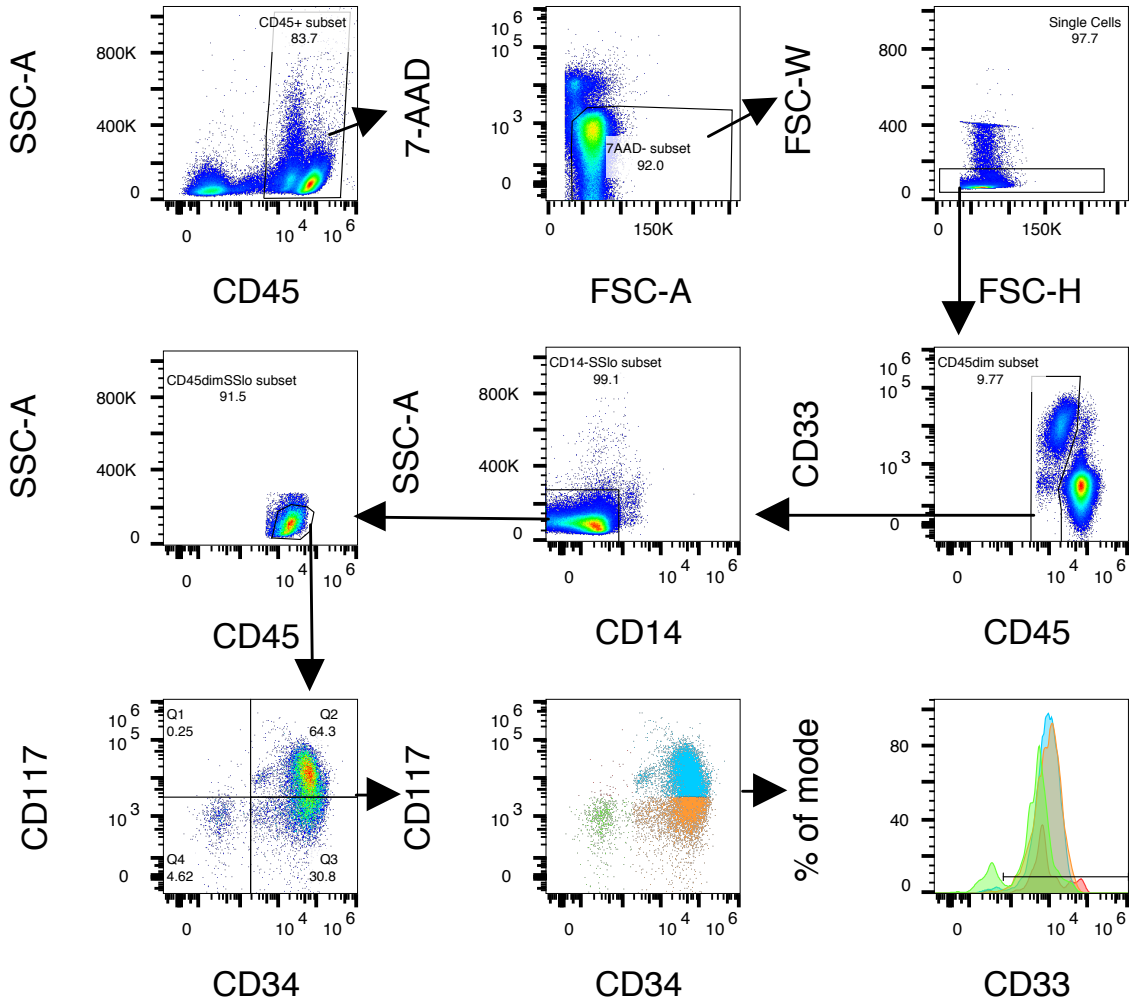
Patient 002-004 Blood C2D2



Patient 002-004 Blood C2D3



Patient 002-004 Blood C2D15



Patient 002-004 BM C2D15

